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**(54) Title:** RECOMBINANT POXVIRUS-FELINE INFECTIOUS PERITONITIS VIRUS, COMPOSITIONS THEREOF AND METHODS FOR MAKING AND USING THEM

**(57) Abstract**

Attenuated recombinant viruses containing DNA encoding FIPV antigen(s), compositions thereof, as well as methods for making and using the compositions, expression products therefrom, and antibodies generated, are disclosed and claimed. The recombinant viruses can be NYVAC or ALVAC recombinant viruses. The compositions and products therefrom and antibodies generated have several preventive, therapeutic and diagnostic uses.

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# RECOMBINANT POXVIRUS-FELINE INFECTIOUS PERITONITIS VIRUS, COMPOSITIONS THEREOF AND METHODS FOR MAKING AND USING THEM

## RELATED APPLICATIONS

5        Reference is made to allowed application Serial No.
08/105,483, filed August 12, 1993, which in turn is a
continuation of application Serial No. 07/847,951, filed
March 6, 1992, which in turn is a continuation-in-part of
10   application Serial No. 07/713,967, filed June 11, 1991,
which in turn is a continuation-in-part of application
Serial No. 07/666,056, filed March 7, 1991, now allowed
application Serial No. 08/036,217, filed March 24, 1993,
and issued November 15, 1994 as U.S. Patent No.
15   5,364,773. Each of the aforementioned and above-
referenced applications and patent are hereby
incorporated herein by reference.

## FIELD OF THE INVENTION

        The present invention relates to modified
20   recombinant poxviruses, compositions thereof and to
methods of making and using the same; for instance, a
vaccinia virus or avipox (e.g. canarypox or fowlpox)
virus. For example, the invention relates to modified
poxvirus-feline infectious peritonitis virus (FIPV)
25   recombinants, compositions thereof, and methods for
making and using the recombinants and compositions. The
invention further relates to such recombinants which are
attenuated recombinants, especially NYVAC- or ALVAC-FIPV
recombinants, compositions thereof and methods for making
30   and using the recombinants and compositions. Thus, the
invention relates to a recombinant poxvirus-FIPV, such
recombinants which express(es) gene product(s) of FIPV,
compositions containing such recombinants and/or gene
product(s), and methods for making and using the
35   recombinants or compositions. The gene product can be
FIPV N, M, and three versions of S (S1-complete spike;
S2-spike minus the signal sequence; and S3-spike C-

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terminal section) or combinations thereof such as M and
N.  The recombinants or compositions containing them can
induce an immunological response against FIPV infection,
when administered to a host.  The host is preferably a
feline, e.g., a cat or kitten.  The response can be
protective.  Thus, the composition can be immunological,
or antigenic, or a vaccine.

The invention additionally relates to the products
of expression of the poxvirus which by themselves are
useful for eliciting an immune response e.g., raising
antibodies or stimulating cell-mediated responses, which
antibodies or responses are useful against FIPV
infection, or which expression products or antibodies
elicited thereby, isolated from a cell culture or from an
animal, are useful for preparing a diagnostic kit, test
or assay for the detection of FIPV, or of the recombinant
virus, or of infected cells, or, of the expression of the
antigens or products in other systems.  The isolated
expression products and antibodies elicited by the
recombinant virus are especially useful in kits, tests or
assays for detection of antibodies or antigens in a
system, host, serum or sample; and the expression
products are useful for generation of antibodies.

Several publications are referenced in this
application.  Full citation to these references is found
at the end of the specification immediately preceding the
claims or where the publication is mentioned; and each of
these publications is hereby incorporated herein by
reference.

**BACKGROUND OF THE INVENTION**

Vaccinia virus and more recently other poxviruses
have been used for the insertion and expression of
foreign genes.  The basic technique of inserting foreign
genes into live infectious poxvirus involves
recombination between pox DNA sequences flanking a
foreign genetic element in a donor plasmid and homologous

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sequences present in the rescuing poxvirus (Piccini et
al., 1987).

Specifically, the recombinant poxviruses are
constructed in two steps known in the art which are
5  analogous to the methods for creating synthetic
recombinants of poxviruses such as the vaccinia virus and
avipox virus described in U.S. Patent Nos. 4,769,330,
4,772,848, 4,603,112, 5,100,587, and 5,179,993, the
disclosures of which are incorporated herein by
10  reference.

First, the DNA gene sequence to be inserted into the
virus, particularly an open reading frame from a non-pox
source, is placed into an *E. coli* plasmid construct into
which DNA homologous to a section of DNA of the poxvirus
15  has been inserted. Separately, the DNA gene sequence to
be inserted is ligated to a promoter. The promoter-gene
linkage is positioned in the plasmid construct so that
the promoter-gene linkage is flanked on both ends by DNA
homologous to a DNA sequence flanking a region of pox DNA
20  containing a nonessential locus. The resulting plasmid
construct is then amplified by growth within *E. coli*
bacteria (Clewell, 1972) and isolated (Clewell et al.,
1969; Maniatis et al., 1982).

Second, the isolated plasmid containing the DNA gene
25  sequence to be inserted is transfected into a cell
culture, e.g. chick embryo fibroblasts, along with the
poxvirus. Recombination between homologous pox DNA in
the plasmid and the viral genome respectively gives a
poxvirus modified by the presence, in a nonessential
30  region of its genome, of foreign DNA sequences. The term
"foreign" DNA designates exogenous DNA, particularly DNA
from a non-pox source, that codes for gene products not
ordinarily produced by the genome into which the
exogenous DNA is placed.

35  Genetic recombination is in general the exchange of
homologous sections of DNA between two strands of DNA.
In certain viruses RNA may replace DNA. Homologous

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sections of nucleic acid are sections of nucleic acid
(DNA or RNA) which have the same sequence of nucleotide
bases.

    Genetic recombination may take place naturally
5   during the replication or manufacture of new viral
genomes within the infected host cell. Thus, genetic
recombination between viral genes may occur during the
viral replication cycle that takes place in a host cell
which is co-infected with two or more different viruses
10  or other genetic constructs. A section of DNA from a
first genome is used interchangeably in constructing the
section of the genome of a second co-infecting virus in
which the DNA is homologous with that of the first viral
genome.

15      However, recombination can also take place between
sections of DNA in different genomes that are not
perfectly homologous. If one such section is from a
first genome homologous with a section of another genome
except for the presence within the first section of, for
20  example, a genetic marker or a gene coding for an
antigenic determinant inserted into a portion of the
homologous DNA, recombination can still take place and
the products of that recombination are then detectable by
the presence of that genetic marker or gene in the
25  recombinant viral genome. Additional strategies have
recently been reported for generating recombinant
vaccinia virus.

    Successful expression of the inserted DNA genetic
sequence by the modified infectious virus requires two
30  conditions. First, the insertion must be into a
nonessential region of the virus in order that the
modified virus remain viable. The second condition for
expression of inserted DNA is the presence of a promoter
in the proper relationship to the inserted DNA. The
35  promoter must be placed so that it is located upstream
from the DNA sequence to be expressed.

Vaccinia virus has been used successfully to immunize against smallpox, culminating in the worldwide eradication of smallpox in 1980. In the course of its history, many strains of vaccinia have arisen. These

5 different strains demonstrate varying immunogenicity and are implicated to varying degrees with potential complications, the most serious of which are post-vaccinial encephalitis and generalized vaccinia (Behbehani, 1983).

10 With the eradication of smallpox, a new role for vaccinia became important, that of a genetically engineered vector for the expression of foreign genes. Genes encoding a vast number of heterologous antigens have been expressed in vaccinia, often resulting in

15 protective immunity against challenge by the corresponding pathogen (reviewed in Tartaglia et al., 1990a, 1990b).

The genetic background of the vaccinia vector has been shown to affect the protective efficacy of the

20 expressed foreign immunogen. For example, expression of Epstein Barr Virus (EBV) gp340 in the Wyeth vaccine strain of vaccinia virus did not protect cottontop tamarins against EBV virus induced lymphoma, while expression of the same gene in the WR laboratory strain

25 of vaccinia virus was protective (Morgan et al., 1988).

A fine balance between the efficacy and the safety of a vaccinia virus-based recombinant vaccine candidate is extremely important. The recombinant virus must present the immunogen(s) in a manner that elicits a

30 protective immune response in the vaccinated animal but lacks any significant pathogenic properties. Therefore attenuation of the vector strain would be a highly desirable advance over the current state of technology.

A number of vaccinia genes have been identified

35 which are non-essential for growth of the virus in tissue culture and whose deletion or inactivation reduces virulence in a variety of animal systems.

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The gene encoding the vaccinia virus thymidine
kinase (TK) has been mapped (Hruby et al., 1982) and
sequenced (Hruby et al., 1983; Weir et al., 1983).
Inactivation or complete deletion of the thymidine kinase
5   gene does not prevent growth of vaccinia virus in a wide
variety of cells in tissue culture. TK⁻ vaccinia virus
is also capable of replication *in vivo* at the site of
inoculation in a variety of hosts and administered by a
variety of routes.

10      It has been shown for herpes simplex virus type 2
that intravaginal inoculation of guinea pigs with TK⁻
virus resulted in significantly lower virus titers in the
spinal cord than did inoculation with TK⁺ virus
(Stanberry et al., 1985). It has been demonstrated that
15  herpesvirus encoded TK activity *in vitro* was not
important for virus growth in actively metabolizing
cells, but was required for virus growth in quiescent
cells (Jamieson et al., 1974).

Attenuation of TK⁻ vaccinia has been shown in mice
20  inoculated by the intracerebral and intraperitoneal
routes (Buller et al., 1985). Attenuation was observed
both for the WR neurovirulent laboratory strain and for
the Wyeth vaccine strain. In mice inoculated by the
intradermal route, TK⁻ recombinant vaccinia generated
25  equivalent anti-vaccinia neutralizing antibodies as
compared with the parental TK⁺ vaccinia virus, indicating
that in this test system the loss of TK function does not
significantly decrease immunogenicity of the vaccinia
virus vector. Following intranasal inoculation of mice
30  with TK⁻ and TK⁺ recombinant vaccinia virus (WR strain),
significantly less dissemination of virus to other
locations, including the brain, has been found (Taylor et
al., 1991a).

Another enzyme involved with nucleotide metabolism
35  is ribonucleotide reductase. Loss of virally encoded
ribonucleotide reductase activity in herpes simplex virus
(HSV) by deletion of the gene encoding the large subunit

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was shown to have no effect on viral growth and DNA
synthesis in dividing cells *in vitro*, but severely
compromised the ability of the virus to grow on serum
starved cells (Goldstein et al., 1988). Using a mouse
5    model for acute HSV infection of the eye and
reactivatable latent infection in the trigeminal ganglia,
reduced virulence was demonstrated for HSV deleted of the
large subunit of ribonucleotide reductase, compared to
the virulence exhibited by wild type HSV (Jacobson et
10   al., 1989).

Both the small (Slabaugh et al., 1988) and large
(Schmidtt et al., 1988) subunits of ribonucleotide
reductase have been identified in vaccinia virus.
Insertional inactivation of the large subunit of
15   ribonucleotide reductase in the WR strain of vaccinia
virus leads to attenuation of the virus as measured by
intracranial inoculation of mice (Child et al., 1990).

The vaccinia virus hemagglutinin gene (HA) has been
mapped and sequenced (Shida, 1986). The HA gene of
20   vaccinia virus is nonessential for growth in tissue
culture (Ichihashi et al., 1971). Inactivation of the HA
gene of vaccinia virus results in reduced neurovirulence
in rabbits inoculated by the intracranial route and
smaller lesions in rabbits at the site of intradermal
25   inoculation (Shida et al., 1988). The HA locus was used
for the insertion of foreign genes in the WR strain
(Shida et al., 1987), derivatives of the Lister strain
(Shida et al., 1988) and the Copenhagen strain (Guo et
al., 1989) of vaccinia virus. Recombinant HA⁻ vaccinia
30   virus expressing foreign genes have been shown to be
immunogenic (Guo et al., 1989; Itamura et al., 1990;
Shida et al., 1988; Shida et al., 1987) and protective
against challenge by the relevant pathogen (Guo et al.,
1989; Shida et al., 1987).

35   Cowpox virus (Brighton red strain) produces red
(hemorrhagic) pocks on the chorioallantoic membrane of
chicken eggs. Spontaneous deletions within the cowpox

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genome generate mutants which produce white pocks (Pickup
et al., 1984).  The hemorrhagic function (u) maps to a 38
kDa protein encoded by an early gene (Pickup et al.,
1986).  This gene, which has homology to serine protease
5   inhibitors, has been shown to inhibit the host
inflammatory response to cowpox virus (Palumbo et al.,
1989) and is an inhibitor of blood coagulation.

          The u gene is present in WR strain of vaccinia virus
(Kotwal et al., 1989b).  Mice inoculated with a WR
10   vaccinia virus recombinant in which the u region has been
inactivated by insertion of a foreign gene produce higher
antibody levels to the foreign gene product compared to
mice inoculated with a similar recombinant vaccinia virus
in which the u gene is intact (Zhou et al., 1990).  The u
15   region is present in a defective nonfunctional form in
Copenhagen strain of vaccinia virus (open reading frames
B13 and B14 by the terminology reported in Goebel et al.,
1990a,b).

          Cowpox virus is localized in infected cells in
20   cytoplasmic A type inclusion bodies (ATI) (Kato et al.,
1959).  The function of ATI is thought to be the
protection of cowpox virus virions during dissemination
from animal to animal (Bergoin et al., 1971).  The ATI
region of the cowpox genome encodes a 160 kDa protein
25   which forms the matrix of the ATI bodies (Funahashi et
al., 1988; Patel et al., 1987).  Vaccinia virus, though
containing a homologous region in its genome, generally
does not produce ATI.  In WR strain of vaccinia; the ATI
region of the genome is translated as a 94 kDa protein
30   (Patel et al., 1988).  In Copenhagen strain of vaccinia
virus, most of the DNA sequences corresponding to the ATI
region are deleted, with the remaining 3' end of the
region fused with sequences upstream from the ATI region
to form open reading frame (ORF) A26L (Goebel et al.,
35   1990a,b).

          A variety of spontaneous (Altenburger et al., 1989;
Drillien et al., 1981; Lai et al., 1989; Moss et al.,

1981; Paez et al., 1985; Panicali et al., 1981) and
engineered (Perkus et al., 1991; Perkus et al., 1989;
Perkus et al., 1986) deletions have been reported near
the left end of the vaccinia virus genome. A WR strain

5   of vaccinia virus with a 10 kb spontaneous deletion (Moss
et al., 1981; Panicali et al., 1981) was shown to be
attenuated by intracranial inoculation in mice (Buller et
al., 1985). This deletion was later shown to include 17
potential ORFs (Kotwal et al., 1988b). Specific genes

10  within the deleted region include the virokine N1L and a
35 kDa protein (C3L, by the terminology reported in
Goebel et al., 1990a,b). Insertional inactivation of N1L
reduces virulence by intracranial inoculation for both
normal and nude mice (Kotwal et al., 1989a). The 35 kDa

15  protein is secreted like N1L into the medium of vaccinia
virus infected cells. The protein contains homology to
the family of complement control proteins, particularly
the complement 4B binding protein (C4bp) (Kotwal et al.,
1988a). Like the cellular C4bp, the vaccinia 35 kDa

20  protein binds the fourth component of complement and
inhibits the classical complement cascade (Kotwal et al.,
1990). Thus the vaccinia 35 kDa protein appears to be
involved in aiding the virus in evading host defense
mechanisms.

25      The left end of the vaccinia genome includes two
genes which have been identified as host range genes, K1L
(Gillard et al., 1986) and C7L (Perkus et al., 1990).
Deletion of both of these genes reduces the ability of
vaccinia virus to grow on a variety of human cell lines

30  (Perkus et al., 1990).
        Two additional vaccine vector systems involve the
use of naturally host-restricted poxviruses, avipox
viruses. Both fowlpoxvirus (FPV) and canarypoxvirus
(CPV) have been engineered to express foreign gene

35  products. Fowlpox virus (FPV) is the prototypic virus of
the Avipox genus of the Poxvirus family. The virus
causes an economically important disease of poultry which

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has been well controlled since the 1920's by the use of
live attenuated vaccines. Replication of the avipox
viruses is limited to avian species (Matthews, 1982) and
there are no reports in the literature of avipoxvirus
5    causing a productive infection in any non-avian species
including man. This host restriction provides an
inherent safety barrier to transmission of the virus to
other species and makes use of avipoxvirus based vaccine
vectors in veterinary and human applications an
10   attractive proposition.

FPV has been used advantageously as a vector
expressing antigens from poultry pathogens. The
hemagglutinin protein of a virulent avian influenza virus
was expressed in an FPV recombinant (Taylor et al.,
15   1988a). After inoculation of the recombinant into
chickens and turkeys, an immune response was induced
which was protective against either a homologous or a
heterologous virulent influenza virus challenge (Taylor
et al., 1988a). FPV recombinants expressing the surface
20   glycoproteins of Newcastle Disease Virus have also been
developed (Taylor et al., 1990; Edbauer et al., 1990).

Despite the host-restriction for replication of FPV
and CPV to avian systems, recombinants derived from these
viruses were found to express extrinsic proteins in cells
25   of nonavian origin. Further, such recombinant viruses
were shown to elicit immunological responses directed
towards the foreign gene product and where appropriate
were shown to afford protection from challenge against
the corresponding pathogen (Tartaglia et al., 1993a,b;
30   Taylor et al., 1992; 1991b; 1988b).

Feline infectious peritonitis virus (FIPV) produces
a chronic, progressive, immunologically-mediated disease
in felines such as domestic and exotic cats. The route
of FIPV infection is thought to occur primarily through
35   the oral cavity and pharynx. Clinically apparent FIP
occurs after the virus crosses the mucosal barrier and a
primary viremia takes FIPV to its many target organs

(liver, spleen, intestine and lungs). Two forms of the
disease have been described as effusive (wet) and non-
effusive (dry). The effusive form results in the classic
fluid accumulation seen in infected cats which is caused
5    by an Arthus-type vasculitis in the target organs
mediated by complement activation and an intense
inflammatory response. The non-effusive form is
characterized by little or no ascitic fluid accumulation
but internal organs may be infiltrated with granular
10   fibrinous deposits. Thus, antibodies formed in response
to FIPV infection (primarily to the spike protein) tend
to enhance the pathogenesis of the disease and are
obviously unwanted in a vaccine or immunological
composition (Olsen and Scott, 1991). (However,
15   expression of such proteins by a recombinant and the
recombinants themselves are useful if one desires
antigens or antibodies therefrom for a kit, test or assay
or the like).

FIPV is a member of the *Coronaviridae* family.
*Coronaviruses* are large, positive stranded RNA viruses
20   with genomic lengths of 27-30 kb. The virion is
enveloped and is studded with peplomeric structures
called spikes. The left half of the FIPV genome encodes
a large polyprotein which is cleaved into smaller
25   fragments, some of which are involved in RNA replication.
The right half of the FIPV genome encodes 3 major
structural proteins designated nucleocapsid (N), matrix
(M) and spike (S). The FIPV S gene product mediates
attachment of the virus to the cell receptor, triggers
30   membrane fusion, and elicits virus-neutralizing
antibodies. The N protein is necessary for encapsidating
genomic RNA and directing its incorporation into the
capsid, and is thought to be involved in RNA replication.
The FIPV M glycoprotein appears to be important for FIP
35   viral maturation and for the determination of the site at
which virus particles are assembled (Spann et al., 1988).

Because of the antibody-dependent enhancement (ADE)
of FIP in cats, attempts to produce a safe and

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efficacious vaccine or immunological composition against
FIPV have been largely unsuccessful.  Inactivated FIPV
vaccines and heterologous live coronavirus vaccines did
not afford any protection against FIPV infection and
5   vaccination usually resulted in increased sensitization
to the disease.  A modified live virus vaccine,
Primucell, is the first and only commercially marketed
FIPV vaccine.  Primucell is a temperature sensitive
strain of FIPV that can replicate at the cooler
10  temperatures of the nasal cavity, but not at systemic
body temperatures (Gerber et al., 1990).  Thus,
intranasally administered Primucell is thought to produce
a localized immunity to FIPV.  However, serious questions
remain concerning the efficacy and enhancement potential
15  of this vaccine (Olsen and Scott, 1991).

Vaccinia virus has been used as a vector for
generating recombinant viruses expressing FIPV structural
genes.  A recombinant expressing the FIP M gene was shown
to increase the survival time of cats after challenge
20  with FIPV (Vennema et al., 1990).

Vennema, et al. (1991) relates to primary structure
of the membrane and nucleocapsid protein genes of feline
infectious peritonitis virus and to certain recombinant
vaccinia viruses thereof introduced into kittens.  The
25  Vennema et al. FIPV matrix gene was cloned from a
pathogenic strain (79-1146) and its sequence appears
identical to the matrix gene (discussed herein).  The
Vennema et al. recombinant, vFM, contains the coding
region of matrix coupled to the vaccinia 7.5K early/late
30  promoter inserted at the thymidine kinase (tk) locus.
Note that the promotor was not linked precisely to the
matrix ATG initiation codon, but rather to a position 48
bp upstream from the ATC.  Also, a vaccinia T5NT early
transcriptional termination signal (Yuen et al., 1987)
35  located in the coding region of the matrix gene was not
removed.

Moreover, the vaccinia strain in Vennema et al. is
the WR strain (Vennema et al. at page 328, left column,

first 2 lines; see also, the donor plasmids and control
viruses as mentioned on the same page in the section
"Construction of Recombinant Vaccinia Viruses expressing
the FIPV M and N proteins" beginning at mid-left column

5    clearly indicate via literature citations that the WR
strain is used). The choice of strain is important
because the WR strain is a laboratory virus - not a
vaccine strain - and the virulence characteristics of the
WR strain do not make it a presently acceptable vector

10   for a recombinant that may contact humans, let alone a
recombinant in a composition such as a vaccine or
antigenic or immunological composition targeted to
felines, such as kittens, or other animals in contact
with humans, especially young children or

15   immunosuppressed individuals, due to recent concerns of
contact transmission (such "other animals" could be
laboratory cell cultures or animals for antigen
expression or for antibody production for making kits,
tests or assays).

20       Thus, the Vennema, et al. articles fail to teach or
suggest the recombinants, compositions and methods of the
present invention.

More particularly, recombinants in the present
invention preferably employ NYVAC or vectors (NYVAC and

25   ALVAC are highly attenuated vectors having a BSL1
containment level).

Further, in constructs of the present invention,
preferably the coding region is coupled to the promotor
in a precise coupling to the ATG codon with no

30   intervening sequence. (Any T5NT sequence can be
inactivated by a base substitution which does not change
the amino acid sequence but will prevent early
transcriptional termination in a poxvirus vector). In
addition, multiple, e.g., two, copies of the coding

35   region directly coupled to the promotor can be present in
each recombinant viral genome in the present invention.

The Vennema et al. efficacy study used SPF kittens
(13-14 weeks old) which were vaccinated subcutaneously at

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day 0 and day 21 with 1 x 10$^8$ and 5 x 10$^8$ pfu
respectively.  On day 35 the cats were challenged orally
with FIP strain 79-1146.

The herein protocol was similar, with the major
5   difference being a lower vaccination dose (1 x 10$^7$).  The
Vennema protection results were based on mortality with 3
of 8 cats vaccinated with vFM surviving (37.5%).  Vennema
et al. deemed their challenge sufficient in that 7 of 8
unvaccinated cats succumbed to the challenge exposure and
10  died.  Upon necropsy, <u>all</u> challenged cats, in Vennema et
al. <u>including the three surviving vFM vaccinated cats</u>,
had pathological signs of FIP infection including
peritoneal effusions and granulomatous lesions on the
viscera.
15  By contrast, the trials herein were more stringent.
Herein applicants scored protection <u>as surviving and</u>
<u>being free from FIP pathology upon necropsy</u>.  Using this
criteria, Applicants had 3 out of 5 cats vaccinated with
vCP262 protected (60%) with 0% of the unvaccinated cats
20  protected.  If the Vennema et al. results were scored
using Applicants' criteria, Vennema would have had no
protection; and ergo no recombinant suitable for vaccine
use.  In addition, the Vennema et al. observed fever and
weight loss in all challenged cats.  In Applicants'
25  trials, (see trial 3 in particular) Applicants' observed
even no weight loss and a lower febrile response after
challenge.

Thus, the recombinants of the present invention
employ an acceptable vector for all uses and a
30  surprisingly higher protection level at a lower dose than
the Vennema et al. vaccinia recombinant.

Recent studies using monoclonal antibodies directed
against the S gene (Olsen et al., 1992) have shown also
that mABs which neutralize the virus also cause ADE.  No
35  enhancement is observed with mABs against matix or
nucleocapsid proteins.

Thus, prior to the present invention, there has been
a need for poxvirus-FIPV recombinants, especially such

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recombinants using an acceptable vector and such
recombinants having expression at low doses which indeed
affords protection; and, there has been a need for
compositions containing such recombinants, as well as a
5   need for methods for making and using them.  And,
moreover, it would be especially surprising and
unexpected if this poxvirus-FIPV recombinant was modified
so as to be attenuated, e.g., an attenuated vaccinia
virus-FIPV recombinant or an attenuated avipox-FIPV
10  recombinant, such as a NYVAC-FIPV or ALVAC-FIPV
recombinant; because, for instance, from attenuation and,
diminished or lack of productive replication of the
poxvirus in the host, one skilled in the art would have
not expected and would be surprised by the usefulness of
15  the attenuated recombinant, especially in a composition
for felines and other hosts, and more especially in such
a composition which provides a response including
protection in felines.

Attenuated poxvirus vectors would also be especially
20  advantageous for antigenic or vaccine compositions,
particularly in view of attenuated vectors providing
diminished or little or no pathogenic properties with
regard to the intended host or, to unintended, possibly
accidental hosts, such as those who work with the vector
25  in formulating or administering the vector or antigen, or
who may otherwise come into contact with it.  That is,
attenuated poxvirus vectors provide diminished or little
or no pathogenic properties to intended hosts such as
cats, kittens and the like and to unintended, possibly
30  accidental hosts, such as humans engaged in formulating
the vector into a composition for administration or in
administering the composition (e.g., veterinarians,
technicians, other workers) or, who may otherwise come
into contact with the vector (e.g., pet owners).
35       It can thus be appreciated that provision of a FIPV
recombinant poxvirus, and of compositions and products
therefrom, particularly NYVAC or ALVAC based FIPV
recombinants and compositions and products therefrom,

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would be a highly desirable advance over the current
state of technology.

## OBJECTS AND SUMMARY OF THE INVENTION

It is therefore an object of this invention to
5    provide modified recombinant viruses, which viruses have
enhanced safety, and to provide a method of making such
recombinant viruses.

Additional objects of this invention include:  to
provide a recombinant poxvirus-FIPV, compositions
10   containing the recombinant, antigen(s) from the
recombinant or from the composition, methods for making
the recombinant and composition, methods of using the
compositions or the recombinant, e.g., in vivo and in
vitro uses for expression by administering or infecting.
15   Preferably the poxvirus-FIPV recombinant composition is
an antigenic, or vaccine or immunological composition
(i.e., a composition containing a recombinant which
expresses antigen, or the product from expression of the
antigen).

20        It is a further object of this invention to provide
a modified vector for expressing a gene product in a
host, wherein the vector is modified so that it has
attenuated virulence in the host.

It is another object of this invention to provide a
25   method for expressing a gene product in a cell cultured
in vitro using a modified recombinant virus or modified
vector having an increased level of safety and to provide
the use of such product in compositions.

In one aspect, the present invention relates to a
30   modified recombinant virus having inactivated virus-
encoded genetic functions so that the recombinant virus
has attenuated virulence and enhanced safety.  The
functions can be non-essential, or associated with
virulence.  The virus is advantageously a poxvirus,
35   particularly a vaccinia virus or an avipox virus, such as
fowlpox virus and canarypox virus.  The modified
recombinant virus can include, within a non-essential

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region of the virus genome, a heterologous DNA sequence
which encodes an antigen or epitope derived from FIPV.

In another aspect, the present invention relates to
an antigenic, immunological or vaccine composition or a
5    therapeutic composition for inducing an antigenic or
immunological or protective response in a host animal
inoculated with the composition, said composition
including a carrier and a modified recombinant virus
having inactivated nonessential virus-encoded genetic
10   functions so that the recombinant virus has attenuated
virulence and enhanced safety.  The virus used in the
composition according to the present invention is
advantageously a poxvirus, particularly a vaccinia virus
or an avipox virus, such as fowlpox virus and canarypox
15   virus.  The modified recombinant virus can include,
within a non-essential region of the virus genome, a
heterologous DNA sequence which encodes an antigenic
protein, e.g., derived from FIPV.  The composition can
contain a recombinant poxvirus which contains coding for
20   and expresses FIPV antigen(s) or the isolated antigen(s).

In yet another aspect, the present invention relates
to methods employing the aforementioned recombinant or
composition; for instance, for obtaining an *in vivo*
response to FIPV antigen(s).  The method can comprise
25   administering the recombinant or composition either to
felines or other hosts, e.g., laboratory animals such as
rodents such as rats, mice, gerbils or the like for
antibody production for kits, assays and the like.

In a further aspect, the present invention relates
30   to a method for expressing a gene product in a cell *in
vitro* by introducing into the cell a modified recombinant
virus having attenuated virulence and enhanced safety.
The modified recombinant virus can include, within a
nonessential region of the virus genome, a heterologous
35   DNA sequence which encodes an antigenic protein, e.g.
derived from FIPV virus.  The product can then be
administered to individuals, e.g., felines or mice to
stimulate an immune response.  The antibodies raised can

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be useful in individuals for the prevention or treatment
of FIPV or and, the antibodies from individuals or
animals or the isolated *in vitro* expression products can
be used in diagnostic kits, assays or tests to determine
5    the presence or absence in a sample such as sera of
rabies or other maladies or antigens therefrom or
antibodies thereto (and therefore the absence or presence
of the virus or of the products, or of an immune response
to the virus or antigens).

10       In a still further aspect, the present invention
relates to a modified recombinant virus and compositions
containing such.  The virus can have nonessential virus-
encoded genetic functions inactivated therein so that the
virus has attenuated virulence, and the modified
15   recombinant virus further contains DNA from a
heterologous source in a nonessential region of the virus
genome.  The DNA can code for FIPV antigen(s).  In
particular, the genetic functions are inactivated by
deleting an open reading frame encoding a virulence
20   factor or by utilizing naturally host restricted viruses.
The virus used according to the present invention is
advantageously a poxvirus, particularly a vaccinia virus
or an avipox virus, such as fowlpox virus and canarypox
virus.  Advantageously, the open reading frame is
25   selected from the group consisting of J2R, B13R + B14R,
A26L, A56R, C7L - K1L, and I4L (by the terminology
reported in Goebel et al., 1990a,b); and, the combination
thereof.  In this respect, the open reading frame
comprises genomic regions which comprise a thymidine
30   kinase gene, a hemorrhagic region, an A type inclusion
body region, a hemagglutinin gene, a host range gene
region or a large subunit, ribonucleotide reductase; or,
the combination thereof.  A suitable modified Copenhagen
strain of vaccinia virus is identified as NYVAC
35   (Tartaglia et al., 1992), or a vaccinia virus from which
has been deleted J2R, B13R+B14R, A26L, A56R, C7L-K1l and
I4L or a thymidine kinase gene, a hemorrhagic region, an
A type inclusion body region, a hemagglutinin gene, a

host range region, and a large subunit, ribonucleotide
reductase (See also U.S. Patent No. 5,364,773).
Alternatively, a suitable poxvirus is an ALVAC or, a
canarypox virus (Rentschler vaccine strain) which was
5    attenuated, for instance, through more than 200 serial
passages on chick embryo fibroblasts, a master seed
therefrom was subjected to four successive plaque
purifications under agar from which a plaque clone was
amplified through five additional passages.

10        The invention in yet a further aspect relates to the
product of expression of the inventive poxvirus-FIPV
recombinant and uses therefor, such as to form antigenic,
immunological or vaccine compositions, for administration
to a host, e.g., animals, such as felines, or for
15   administration for protection or response or for
treatment, prevention, diagnosis or testing, and, to
methods employing such compositions.  The FIPV
antigen(s), or the DNA encoding FIPV antigen(s) can code
for M, N, and the three versions of S; S1, S2, S3, or
20   combinations thereof, e.g., M+N.

          The present invention (recombinants, compositions
and methods and uses) finds a basis in the discoveries
that NYVAC and ALVAC recombinants, particularly NYVAC-
and ALVAC-FIPV recombinants, surprisingly have expression
25   despite attenuation, and expression which can confer a
truly protective response in a susceptible host.

          These and other embodiments are disclosed or are
obvious from and encompassed by the follow detailed
description.

30              **BRIEF DESCRIPTION OF THE DRAWINGS**

          The following detailed description, given by way of
example, but not intended to limit the invention solely
to the specific embodiments described, may best be
understood in conjunction with the accompanying drawings,
35   in which:

                    Figure 1   shows the DNA sequence of FIPV matrix gene
                               open reading frame (strain 79-1146);

. Figure 2   shows the DNA sequence of the FIPV matrix
             gene donor plasmid (The modified matrix
             gene coding region is initiated at 2408
             and terminates at 1620; the entomopox 42K
5            promoter starts at 2474; the C5 left arm
             is from 1 to 1549 and the C5 right arm is
             from 2580 to 2989);

   Figure 3  shows the DNA sequence of FIPV nucleocapsid
             gene open reading frame (strain 79-1146);

10 Figure 4  shows the DNA sequence of the FIPV
             nucleocapsid gene donor plasmid (the
             nucleocapsid gene coding region initiates
             at 2101 and terminates at 968; the
             vaccinia I3L promoter starts at 2160; the
15           C3 left arm is from 1 to 939 and the C3
             right arm is from 2285 to 4857);

   Figure 5  shows the DNA sequence of FIPV spike gene
             open reading frame (strain 79-1146);

   Figure 6  shows the DNA sequence of the FIPV spike
20           gene donor plasmid (the modified spike
             gene coding region is initiated at 591 and
             terminates at 4976; the vaccinia H6
             promoter starts at 471; the C6 left arm is
             from 1 to 387 and the C6 right arm is from
25           4983 to 6144);

   Figure 7  shows the DNA sequence of the FIPV spike
             gene minus signal sequence donor plasmid
             (the modified spike gene coding region is
             initiated at 591 and terminates at 4922;
30           the vaccinia H6 promoter starts at 471;
             the C6 left arm is from 1 to 387 and the
             C6 right arm is from 4929 to 6090);

   Figure 8  shows the DNA sequence of the FIPV spike
             gene C-terminal fragment donor plasmid
35           (the modified spike gene coding region
             initiates at 591 and terminates at 2369;
             the vaccinia H6 promoter starts at 471;

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the C6 left arm is from 1 to 387 and the
C6 right arm is from 2376 to 3537);

Figure 9 shows the DNA sequence of a 7351 bp
fragment of canarypox DNA containing the
C3 open reading frame (the C3 ORF is
initiated at position 1458 and terminates
at position 2897);

Figure 10 shows the DNA sequence of a 3208 bp
fragment of canarypox DNA containing the
C5 open reading frame (the C5 ORF is
initiated at position 1537 and terminates
at position 1857); and,

Figure 11 shows the DNA sequence of a 3706 bp
fragment of canarypox DNA containing the
C6 open reading frame (the C6 ORF is
initiated at position 377 and terminates
at position 2254).

## DETAILED DESCRIPTION OF THE INVENTION

To develop a new vaccinia vaccine strain, NYVAC
(vP866), the Copenhagen vaccine strain of vaccinia virus
was modified by the deletion of six nonessential regions
of the genome encoding known or potential virulence
factors. The sequential deletions are detailed below
(See U.S. Patent No. 5,364,773). All designations of
vaccinia restriction fragments, open reading frames and
nucleotide positions are based on the terminology
reported in Goebel et al., 1990a,b.

The deletion loci were also engineered as recipient
loci for the insertion of foreign genes.

The regions deleted in NYVAC are listed below. Also
listed are the abbreviations and open reading frame
designations for the deleted regions (Goebel et al.,
1990a,b) and the designation of the vaccinia recombinant
(vP) containing all deletions through the deletion
specified:

(1) thymidine kinase gene (TK; J2R) vP410;

(2) hemorrhagic region (u; B13R + B14R) vP553;

(3) A type inclusion body region (ATI; A26L) vP618;

     (4)  hemagglutinin gene (HA; A56R) vP723;

     (5)  host range gene region (C7L - K1L) vP804; and

     (6)  large subunit, ribonucleotide reductase (I4L)
         vP866 (NYVAC).

5        NYVAC is a genetically engineered vaccinia virus
strain that was generated by the specific deletion of
eighteen open reading frames encoding gene products
associated with virulence and host range. NYVAC is
highly attenuated by a number of criteria including i)
10  decreased virulence after intracerebral inoculation in
newborn mice, ii) inocuity in genetically (nu⁺/nu⁺) or
chemically (cyclophosphamide) immunocompromised mice,
iii) failure to cause disseminated infection in
immunocompromised mice, iv) lack of significant
15  induration and ulceration on rabbit skin, v) rapid
clearance from the site of inoculation, and vi) greatly
reduced replication competency on a number of tissue
culture cell lines including those of human origin.
Nevertheless, NYVAC based vectors induce excellent
20  responses to extrinsic immunogens and provided protective
immunity.

       TROVAC refers to an attenuated fowlpox that was a
plaque-cloned isolate derived from the FP-1 vaccine
strain of fowlpoxvirus which is licensed for vaccination
25  of chicks. ALVAC is an attenuated canarypox virus-based
vector that was a plaque-cloned derivative of the
licensed canarypox vaccine, Kanapox (Tartaglia et al.,
1992). ALVAC has some general properties which are the
same as some general properties of Kanapox. ALVAC-based
30  recombinant viruses expressing extrinsic immunogens have
also been demonstrated efficacious as vaccine vectors
(Tartaglia et al., 1993a,b). This avipox vector is
restricted to avian species for productive replication.
On human cell cultures, canarypox virus replication is
35  aborted early in the viral replication cycle prior to
viral DNA synthesis. Nevertheless, when engineered to
express extrinsic immunogens, authentic expression and
processing is observed *in vitro* in mammalian cells and

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inoculation into numerous mammalian species induces antibody and cellular immune responses to the extrinsic immunogen and provides protection against challenge with the cognate pathogen (Taylor et al., 1992; Taylor et al., 5  1991b). Recent Phase I clinical trials in both Europe and the United States of a canarypox/rabies glycoprotein recombinant (ALVAC-RG) demonstrated that the experimental vaccine was well tolerated and induced protective levels of rabiesvirus neutralizing antibody titers (Cadoz et 10  al., 1992; Fries et al., 1992). Additionally, peripheral blood mononuclear cells (PBMCs) derived from the ALVAC-RG vaccinates demonstrated significant levels of lymphocyte proliferation when stimulated with purified FIPV (Fries et al., 1992).

15      NYVAC, ALVAC and TROVAC have also been recognized as unique among all poxviruses in that the National Institutes of Health ("NIH")(U.S. Public Health Service), Recombinant DNA Advisory Committee, which issues guidelines for the physical containment of genetic 20  material such as viruses and vectors, i.e., guidelines for safety procedures for the use of such viruses and vectors which are based upon the pathogenicity of the particular virus or vector, granted a reduction in physical containment level: from BSL2 to BSL1. No other 25  poxvirus has a BSL1 physical containment level. Even the Copenhagen strain of vaccinia virus - the common smallpox vaccine - has a higher physical containment level; namely, BSL2. Accordingly, the art has recognized that NYVAC, ALVAC and TROVAC have a lower pathogenicity than 30  any other poxvirus.

        Clearly based on the attenuation profiles of the NYVAC, ALVAC, and TROVAC vectors and their demonstrated ability to elicit both humoral and cellular immunological responses to extrinsic immunogens (Tartaglia et al., 35  1993a,b; Taylor et al., 1992; Konishi et al., 1992) such recombinant viruses offer a distinct advantage over previously described vaccinia-based recombinant viruses.

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The invention provides poxvirus-FIPV recombinants,
preferably NYVAC- and ALVAC-FIPV recombinants which
contain exogenous DNA coding for any or all of FIPV, M,
N, and the three versions of S; S1, S2, S3, or
5    combinations thereof, e.g., M+N.

The administration procedure for recombinant
poxvirus-FIPV or expression product thereof, compositions
of the invention such as immunological, antigenic or
vaccine compositions or therapeutic compositions, can be
10   via a parenteral route (intradermal, intramuscular or
subcutaneous). Such an administration enables a systemic
immune response, or humoral or cell-mediated responses.

More generally, the inventive poxvirus-FIPV
recombinants, antigenic, immunological or vaccine
15   poxvirus-FIPV compositions or therapeutic compositions
can be prepared in accordance with standard techniques
well known to those skilled in the pharmaceutical or
veterinary art. Such compositions can be administered in
dosages and by techniques well known to those skilled in
20   the medical or veterinary arts taking into consideration
such factors as the age, sex, weight, species and
condition of the particular patient, and the route of
administration. The compositions can be administered
alone, or can be co-administered or sequentially
25   administered with compositions, e.g., with "other"
immunological, antigenic or vaccine or therapeutic
compositions thereby providing multivalent or "cocktail"
or combination compositions of the invention and methods
employing them. Again, the ingredients and manner
30   (sequential or co-administration) of administration, as
well as dosages can be determined taking into
consideration such factors as the age, sex, weight,
species and condition of the particular patient, and, the
route of administration. In this regard, reference is
35   made to U.S. Serial No. 08/486,969, filed June 7, 1995,
incorporated herein by reference, and directed to rabies
compositions and combination compositions and uses
thereof.

Examples of compositions of the invention include
liquid preparations for orifice, e.g., oral, nasal, anal,
vaginal, peroral, intragastric, etc., administration such
as suspensions, syrups or elixirs; and, preparations for
5   parenteral, subcutaneous, intradermal, intramuscular or
intravenous administration (e.g., injectable
administration) such as sterile suspensions or emulsions.
In such compositions the recombinant poxvirus or antigens
may be in admixture with a suitable carrier, diluent, or
10  excipient such as sterile water, physiological saline,
glucose or the like. The compositions can also be
lyophilized. The compositions can contain auxiliary
substances such as wetting or emulsifying agents, pH
buffering agents, adjuvants, gelling or viscosity
15  enhancing additives, preservatives, flavoring agents,
colors, and the like, depending upon the route of
administration and the preparation desired. Standard
texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th
edition, 1985, incorporated herein by reference, may be
20  consulted to prepare suitable preparations, without undue
experimentation. Suitable dosages can also be based upon
the examples below.

Further, the products of expression of the inventive
recombinant poxviruses and compositions comprising them
25  can be used directly to stimulate an immune response in
individuals or in animals. Thus, the expression products
can be used in compositions of the invention instead or
in addition to the inventive recombinant poxvirus in the
aforementioned compositions.

30  Additionally, the inventive recombinant poxvirus and
the expression products therefrom and compositions of the
invention stimulate an immune or antibody response in
animals; and therefore, those products are antigens.
From those antibodies or antigens, by techniques well-
35  known in the art, monoclonal antibodies can be prepared
and, those monoclonal antibodies or the antigens, can be
employed in well known antibody binding assays,
diagnostic kits or tests to determine the presence or

absence of particular FIPV antigen(s); and therefore, the
presence or absence of the virus or of the antigen(s) or
to determine whether an immune response to the virus or
antigen(s) has simply been stimulated. Those monoclonal

5    antibodies or the antigens can also be employed in
immunoadsorption chromatography to recover or isolate
FIPV antigen(s) or expression products of the inventive
recombinant poxvirus or compositions of the invention.
          Methods for producing monoclonal antibodies and for

10   uses of monoclonal antibodies, and, of uses and methods
for FIPV antigens - the expression products of the
inventive poxvirus and compositions - are well known to
those of ordinary skill in the art. They can be used in
diagnostic methods, kits, tests or assays, as well as to

15   recover materials by immunoadsorption chromatography or
by immunoprecipitation.
          Monoclonal antibodies are immunoglobulins produced
by hybridoma cells. A monoclonal antibody reacts with a
single antigenic determinant and provides greater

20   specificity than a conventional, serum-derived antibody.
Furthermore, screening a large number of monoclonal
antibodies makes it possible to select an individual
antibody with desired specificity, avidity and isotype.
Hybridoma cell lines provide a constant, inexpensive

25   source of chemically identical antibodies and
preparations of such antibodies can be easily
standardized. Methods for producing monoclonal
antibodies are well known to those of ordinary skill in
the art, e.g., Koprowski, H. et al., U.S. Patent No.

30   4,196,265, issued April 1, 1989, incorporated herein by
reference.
          Uses of monoclonal antibodies are known. One such
use is in diagnostic methods, e.g., David, G. and Greene,
H. U.S. Patent No. 4,376,110, issued March 8, 1983;

35   incorporated herein by reference. Monoclonal antibodies
have also been used to recover materials by
immunoadsorption chromatography, e.g., Milstein, C. 1980,

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Scientific American 243:66, 70, incorporated herein by
reference.

Accordingly, the inventive recombinant poxvirus and
compositions have several herein stated utilities. Other
5 utilities also exist for embodiments of the invention.

A better understanding of the present invention and
of its many advantages will be had from the following
examples, given by way of illustration.

**EXAMPLES**

10      DNA Cloning and Synthesis. Plasmids were
constructed, screened and grown by standard procedures
(Maniatis et al., 1982; Perkus et al., 1985; Piccini et
al., 1987). Restriction endonucleases were obtained from
Bethesda Research Laboratories, Gaithersburg, MD, New
15 England Biolabs, Beverly, MA; and Boehringer Mannheim
Biochemicals, Indianapolis, IN. Klenow fragment of E.
coli polymerase was obtained from Boehringer Mannheim
Biochemicals. BAL-31 exonuclease and phage T4 DNA ligase
were obtained from New England Biolabs. The reagents
20 were used as specified by the various suppliers.

Synthetic oligodeoxyribonucleotides were prepared on
a Biosearch 8750 or Applied Biosystems 380B DNA
synthesizer as previously described (Perkus et al.,
1989). DNA sequencing was performed by the dideoxy-chain
25 termination method (Sanger et al., 1977) using Sequenase
(Tabor et al., 1987) as previously described (Guo et al.,
1989). DNA amplification by polymerase chain reaction
(PCR) for sequence verification (Engelke et al., 1988)
was performed using custom synthesized oligonucleotide
30 primers and GeneAmp DNA amplification Reagent Kit (Perkin
Elmer Cetus, Norwalk, CT) in an automated Perkin Elmer
Cetus DNA Thermal Cycler. Excess DNA sequences were
deleted from plasmids by restriction endonuclease
digestion followed by limited digestion by BAL-31
35 exonuclease and mutagenesis (Mandecki, 1986) using
synthetic oligonucleotides.

Cells, Virus, and Transfection. The origins and
conditions of cultivation of the Copenhagen strain of

vaccinia virus has been previously described (Guo et al.,
1989). Generation of recombinant virus by recombination,
*in situ* hybridization of nitrocellulose filters and
screening for B-galactosidase activity are as previously
5   described (Piccini et al., 1987).

The origins and conditions of cultivation of the
Copenhagen strain of vaccinia virus and NYVAC has been
previously described (Guo et al., 1989; Tartaglia et al.,
1992). Generation of recombinant virus by recombination,
10  *in situ* hybridization of nitrocellulose filters and
screening for B-galactosidase activity are as previously
described (Panicali et al., 1982; Perkus et al., 1989).

NYVAC is prepared by reference to U.S. Patent No.
5,364,773 and allowed U.S. application Serial No.
15  105,483, incorporated herein by reference.

The parental canarypox virus (Rentschler strain) is
a vaccinal strain for canaries. The vaccine strain was
obtained from a wild type isolate and attenuated through
more than 200 serial passages on chick embryo
20  fibroblasts. A master viral seed was subjected to four
successive plaque purifications under agar and one plaque
clone was amplified through five additional passages
after which the stock virus was used as the parental
virus in *in vitro* recombination tests. The plaque
25  purified canarypox isolate is designated ALVAC.

The strain of fowlpox virus (FPV) designated FP-1
has been described previously (Taylor et al., 1988a). It
is an attenuated vaccine strain useful in vaccination of
day old chickens. The parental virus strain Duvette was
30  obtained in France as a fowlpox scab from a chicken. The
virus was attenuated by approximately 50 serial passages
in chicken embryonated eggs followed by 25 passages on
chicken embryo fibroblast cells. The virus was subjected
to four successive plaque purifications. One plaque
35  isolate was further amplified in primary CEF cells and a
stock virus, designated as TROVAC, established.

NYVAC, ALVAC and TROVAC viral vectors and their
derivatives were propagated as described previously

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(Piccini et al., 1987; Taylor et al., 1988a,b). Vero
cells and chick embryo fibroblasts (CEF) were propagated
as described previously (Taylor et al., 1988a,b).

**EXAMPLE 1 - GENERATION OF ALVAC-BASED FIPV**

5                    **RECOMBINANTS**

    1.    Generation of an ALVAC Recombinant Expressing
        the Feline Infectious Peritonitis Virus (FIPV)
        Matrix Glycoprotein Gene Open Reading Frame
        (vCP262).

10

The 79-1146 FIPV strain was obtained from Dr. F.
Scott (Cornell University, Ithaca, NY). Total RNA was
isolated from FIPV infected CRFK cells using the
quanidium isothiocyanate-cesium chloride procedure of

15  Chirgwin, et al., (1979). First strand cDNA was
synthesized using AMV reverse transcriptase and random
oligonucleotide primers (6 mers) by the procedure of
Watson and Jackson (1985), yielding single-stranded cDNA
complementary to the FIPV positive strand mRNA.

20       The matrix gene (M) was amplified by PCR from the
first strand cDNA using oligonucleotide primers RG739
(SEQ ID NO:1) (5'-TAAGAGCTCATGAAGTACATTTTGCT-3') and
RG740 (SEQ ID NO:2) (5'-ATTGGTACCGTTTAGTTACACCATATG-3').
These primers were derived from Genbank sequence COFIPVMN

25  (Accession # X56496)(Vennema et al., 1991). This 800 bp
PCR fragment was digested with Asp718/SacI, gel purified,
and ligated into pBluescript SK+ digested with
Asp718/SacI to yield pBSFIPM. The M gene ORF was
sequenced and is presented in Figure 1 (SEQ ID NO:3).

30       pBSFIPM was transformed into GM48 (dam-) cells, and
plasmid DNA isolated which was demethylated (pBSFIPM-
demeth). A 330 bp PCR fragment was amplified from
pBSFIPM using oligonucleotides RG751 (SEQ ID NO:4) (5'-
TCTGAGCTCTTTATTGGGAAGAATATGATAATATTT-

35  GGGATTTCAAAATTGAAAATATATAATTACAATATAAAATGAAGTACATTTTGCT-
3') and RG752 (SEQ ID NO:5)
(5'CACATGATCAGCATTTTAATGCCATAAACGAGCCAGCTAAA-
TTGTGGTCTGCCATATTG TAACACTGTTATAAATACAATC-3') and
digested with SacI/BclI. This fragment was gel purified

40  and ligated into pBSFIPM (demeth) digested with BclI to

yield pFIPM42K. An 85 bp fragment was generated as a PCR
primer-dimer from oligonucleotides RG749 (SEQ ID NO:6)
(5'-TCCGAGCTCTAATTAATT-AACGAGCAGATAGTCTCGTTCTCGCCCTGCCTG-
3')and RG750 (SEQ ID NO:7) (5'-

5    TACGAGCTCAAGCTTCCCGGGTTAATTAATTAGTCATCAGGCAGGGCGAGAACG-
3'). This fragment was digested with SacI and ligated
into pFIPM42K digested with SacI to yield pFIPM42KVQ.
This plasmid construct contains an expression cassette
consisting of the complete FIPV matrix ORF (with a

10   mutated T5NT early transcriptional stop signal) coupled
to the entomopox 42K promoter (SEQ ID NO:8)
(5'TTTATTGGGAAGAATATGATAATATTTTGGG-
ATTTCAAAATTGAAAATATATAATTACAATATAAA-3'). The T5NT
sequence is modified such that it no longer functions as

15   an early transcription stop signal and no amino acids are
changed. This cassette was excised by digesting
pFIPM42KVQ with Asp718/HindIII and isolated as a 950bp
fragment. The ends of this fragment were blunted using
Klenow polymerase and ligated into the ALVAC C5 locus

20   insertion plasmid pNC5LSP-5, digested with SmaI. The
resulting donor plasmid, pC5FIPM42K, was confirmed by DNA
sequence analysis. It consists of the entomopox 42K
promoter coupled to the FIPV matrix ORF at the ATG
flanked by the left and right arms of the ALVAC C5

25   insertion locus (Figure 2 (SEQ ID NO:9)).

          This donor plasmid, pC5FIPM42K, was used in *in vivo*
recombination (Piccini et al., 1987) with the ALVAC virus
vector to generate the recombinant virus vCP262.

          Immunoprecipitation analysis from a radiolabeled

30   lysate of VERO cells infected with vCP262 using a FIP
matrix specific monoclonal antibody designated 15A9.9
(Olsen et al., 1992) showed expression of a 30 kDa
polypeptide band. This was consistent with the expected
size of the M gene product. In addition, the band

35   comigrated with an immunoprecipitated band from FIPV
infected cells. Fluorescent activated cell sorting
(FACS) analysis using the same monoclonal antibody showed

this expressed protein from vCP262 was localized in the
cytoplasm of the infected cell.

     2.     Generation of an ALVAC Recombinant Expressing
             the FIPV Nucleocapsid Gene Open Reading Frame
5           (vCP261A).

     The FIPV nucleocapsid gene (N) was amplified by PCR
using the first strand cDNA (described in 1 above) as
template and oligonucleotide primers RG741 (SEQ ID NO:10)
10  (5'-TAAGAGCTCATG-GCCACACAGGGACAA-3') and RG742 (SEQ ID
NO:11) (5'-TATGGTACCTTA-GTTCGTAACCTCATC-3'). These
primers were derived from Genbank sequence COFIPVMN
(Accession # X56496)(Vennema et al., 1991). The
resulting 1150 bp fragment was digested with Asp718/SacI
15  and ligated into pBluescript SK+ digested with
Asp718/SacI resulting in pBSFIPN. The N gene ORF was
sequenced and is presented in Figure 3 (SEQ ID NO:12).

     The vaccinia I3L promoter (SEQ ID NO:13) (5'-
TGAGATAAAGTGAAAATATATATCATTATATTACAAAGTACAATTATTTAGGTTTAA
20  TC-3')(Schmitt and Stunnenberg, 1988) was coupled to the
ATG of the N ORF as follows. A 370 bp fragment was
amplified by PCR using pBSFIPN as template and
oligonucleotide primers RG747 (SEQ ID NO:14) (5'-
CATCAGCATGAGGTCCTGTACC-3') and RG748 (SEQ ID NO:15)
25  (5'TAAGAGCTCTGAGATAAAGTGAAAATATATA-
TCATTATATTACAAAGTACAATTATTTAGGTTTAATCATGGCCACACAGGGACAA-
3'). This fragment was digested with SacI/PPuMI and
ligated into pBSFIPN digested with SacI/PPuMI resulting
in pFIPNI3L. An 85 bp fragment was generated as a PCR
30  primer-dimer from oligonucleotides RG749 (SEQ ID NO:6)
(5'-TCCGAGCTCTAATTAATTAACGAGCAGATAGTCTCGTTCTCGCCCTGCCTG-
3') and RG750 (SEQ ID NO:7)(5'-
TACGAGCTCAAGCTTCCCGGGTTAATTAATTAGTCA
TCAGGCAGGGCGAGAACG-3'). This fragment was digested with
35  SacI and ligated into pFIPNI3L digested with SacI to
yield pFIPNI3LVQ. The N gene expression cassette (I3L
promoted N) was excised as a 1300 bp fragment by
digesting pFIPNI3LVQ with Asp718/HindIII. The ends of
this fragment were blunted using Klenow polymerase and

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ligated into the C3 insertion plasmid, pSPCP3LSA (see below), digested with SmaI. The resulting donor plasmid, pC3FIPNI3L, was confirmed by DNA sequence analysis. It consists of the vaccinia I3L promoter coupled to the FIPV

5    N gene ORF flanked by the left and right arms of the ALVAC C3 insertion locus (Figure 4 (SEQ ID NO:16)).

This donor plasmid, pC3FIPNI3L, was used in *in vivo* recombination (Piccini et al., 1987) with the ALVAC virus vector to generate the recombinant virus vCP261A.

10    Immunoprecipitation analysis from a radiolabeled lysate of VERO cells infected with vCP261A using a FIP nucleocapsid specific monoclonal antibody designated 17B7.1 (Olsen et al., 1992) showed expression of a 45 kDa polypeptide band. This was consistent with the expected

15    size of the N gene product. In addition, the band comigrated with an immunoprecipitated band from FIPV infected cells. FACS analysis using the same monoclonal antibody showed this expressed protein from vCP261A was localized in the cytoplasm of the infected cell.

20        3.    Generation of an ALVAC Recombinant Expressing
              both the FIPV Matrix and Nucleocapsid Open
              Reading Frames (vCP282).

Plasmid pC5FIPM42K (Figure 2, SEQ ID NO:9)

25    containing the FIPV matrix gene ORF coupled to the entomopox 42K promoter was used in *in vivo* recombination (Piccini et al., 1987) with the ALVAC-FIP-N recombinant (vCP261A) (described in 2 above) to generate the double recombinant vCP282. This recombinant contains the FIPV M

30    gene ORF (42K promoter) inserted into the C5 locus and the FIPV N gene ORF (I3L promoter) inserted into the C3 locus.

Immunoprecipitation analysis from a radiolabeled lysate of VERO cells infected with vCP282 using a FIP

35    matrix specific monoclonal antibody designated 15A9.9 (Olsen et al., 1992) showed expression of a 30 kDa polypeptide band while using a nucleocapsid specific monoclonal antibody designated 17B7.1 showed expression of a 45 kDa polypeptide band. This was consistent with

the expected size of the M and N gene products
respectively. In addition, both bands comigrated with an
immunoprecipitated bands from FIPV infected cells.
Fluorescent activated cell sorting (FACS) analysis using
the same monoclonal antibodies showed these expressed
proteins from vCP282 were localized in the cytoplasm of
the infected cell.

        4.    Generation of an ALVAC Recombinant Expressing
            the Complete FIPV Spike Glycoprotein Gene ORF
            (vCP281).

The FIPV spike gene (S) was obtained by PCR
amplification from first strand cDNA template (described
in 1 above) in three sections. PCR primers were
synthesized based on Genbank sequence COFIPE2 (Accession
#X06170) (De Groot et al., 1987). The 5' end was
amplified by PCR using oligonucleotide primers JP53 (SEQ
ID NO:17) (5'-CATCATGAGCTCATGATTGTGCTCGTAAC-3') and JP77
(SEQ ID NO:18) (5'-AACAGCCGCTTGTGCGC-3'). The isolated
1630 bp fragment was digested with SacI/HindIII and
ligated into pBluescript SK+ digested with SacI/HindIII
to yield pBSFIP-SA, which was confirmed by DNA sequence
analysis.

The middle section of S was amplified by PCR using
oligonucleotide primers JP84 (SEQ ID NO:19) (5'-
CTTGGTATGAAGCTTAG-3') and JP85 (SEQ ID NO:20) (5'-
GGTGACTTAAAGCTTGC-3'). The isolated 1715 bp fragment was
digested with HindIII and ligated into pBluescript SK+
digested with HindIII. Two clones, pKR5 and pKW13 were
sequenced and found to have errors (based on Genbank
sequence COFIPE2) but in different locations. To correct
these PCR errors, a section of pKW13 was replaced with a
subfragment from pKR5 as follows. PKR5 was digested with
ClaI, blunted with Klenow polymerase, digested with
BstEII and a 750 bp fragment isolated and cloned into
pKR13 digested with SmaI/BstEII. The resulting plasmid,
pBSFIPS-MII, was confirmed by DNA sequence analysis.

The 3' section of S was amplified by PCR using
oligonucleotide primers JP71 (SEQ ID NO:21) (5'-

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TAATGATGCTATACATC-3') and JP90 (SEQ ID NO:22) (5'-
CATCATGGTACCTTAGTGGACATGCACTTT-3'). The isolated 1020 bp
fragment was digested with HinDIII/Asp718 and ligated
into pBluescript SK+ digested with HinDIII/Asp718 to
5    yield pBSFIPS-C, which was confirmed by DNA sequence
analysis.

The complete DNA sequence of the FIPV Spike gene as
derived from the 79-1146 strain cDNA is presented in
Figure 5 (SEQ ID NO:23).

10   The spike ORF contains three T5NT early
transcriptional stop signals. Two were eliminated from
the middle section by introducing mutations via PCR. A
330 bp PCR fragment was amplified from pBSFIPS-MII using
oligonucleotide primers RG757B (SEQ ID NO:24) (5'-
15   CATTAGACTCTGTGACGCCATGTGATGTAA-
GCGCACAAGCGGCTGTTATCGATGGTGCCATAGTTGGAGCTATGACTTCCATTAACA
GT- GAACTGTTAGGCCTAACACATTGGACAACGACACCTAATTTCTATTAC-
3')and RG758B (SEQ ID NO:25) (5'-
CATTAGACTGTAAACCTGCATGTATTCAACTTG-
20   CACAGATATTGTAAAATTTGTAGGTATCGTGACATTACCAGTGCTAATTGGTTGCAC
GT-CTCCGTCAGAATGTGTGACGTTAATAAATACCAAAG-3'), digested
with HgaI/BspMI and cloned into HgaI/BspMI digested
pBSFIPS-MII to yield pMJ5. Sequence analysis of pMJ5
revealed a 33 bp deletion which was corrected by
25   replacing the 250 bp StuI/BspMI fragment with a PCR
fragment amplified from pBSFIPS-MII using oligonucleotide
primers RG758B (SEQ ID NO:25) and JP162 (SEQ ID NO:26)
(5'-GTGAACTGTTAGGCCTAACACA-TTGGACAACGACACCTAATTTCTATTAC-
3'). The isolated fragment was digested with StuI/BspMI
30   and ligated into pMJ5 digested with StuI/BspMI to yield
pNR3. This plasmid had a base change at position 2384
which was corrected using the U.S.E. mutagenesis kit
(Pharmacia) to yield pBSFIPS-MIIDII. This plasmid
contains the middle section of the S gene with changed
35   T5NT sequences and the introduction of new ClaI and StuI
sites while maintaining the correct amino acid sequence.

In order to couple the vaccinia H6 promoter (SEQ ID
NO:27) (5'-

TTCTTTATTCTATACTTAAAAAGTGAAAATAAATACAAAGGTTCTTGA-
GGGTTGTGTTAAATTGAAAGCGAGAAAAAAATAATCATAAATTATTTCATTATCGC
G-ATATCCGTTAAGTTTGTATCGTA-3') (Perkus et al., 1989) to
the ATG of the S gene the following was performed. The

5  3' end of the H6 promoter coupled to the S gene amplified
as a PCR fragment from pBSFIPS-A (5' section of S gene)
using oligonucleotide primers RG755 (SEQ ID NO:28). (5'-
CTTGTATGCATTCATTATTTG-3') and RG756 (SEQ ID NO:29) (5'-
TCCGAGCTCGATATCCGTTAAGTTTGTATCGTAATGATTGTGCTCGTAAC-3').

10  The 100 bp fragment was digested with SacI/NsiI and
ligated to pBSFIPS-A digested with SacI/NsiI to yield
pBSFIPS-AH6.

To remove the T5NT sequence in the 5' section of the
spike gene without altering the amino acid sequence, a

15  350 bp PCR fragment was amplified from pBSFIPS-AH6 using
oligonucleotide primers RG753 (SEQ ID NO:30) (5'-
TCACTGCAGATGTACAATCTG-3') and RG754 (SEQ ID NO:31) (5'-
CAGTATACGATGTGTAAGCAATTGTCCAAAAA-
GCTCCACTAACACCAGTGGTTAAAT-

20  TAAAAGATATACAACCAATAGGAAATGTGCTAAAGAAATTGTAACCATTAATATAGA
AATGG-3'). The fragment was digested with PstI/AccI and
ligated into pBSFIPS-AH6 digested with PstI/AccI to yield
pNJ1.

The 5', middle and 3' ends of the S gene were

25  coupled together to form the complete ORF as follows.
First, the 3' section was excised as a 1000 bp fragment
by digesting pBSFIPS-C with Asp718/HinDIII and ligating
into pNJI (5' section) digested with Asp718/HinDIII
yielding pBSFIPS-A/CH6. The middle section was added by

30  excising a 1700 bp fragment from pBSFIPSMIIDII by
digesting with HinDIII and ligating into pBSFIPS-A/CH6
digested with HinDIII and screened for orientation. The
resulting plasmid, pBSFIPSH6II, contains the complete S
ORF coupled to the 3' end of the H6 promoter with all

35  three T5NT sequences eliminated.

To insert the complete S ORF into a C6 donor
plasmid, a 4.4 kb cassette was excised from pBSFIPSH6II
by digesting with EcoRV/EcoRI and filling in the ends

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with Klenow polymerase. This cassette was ligated into
pJCA070 digested with EcoRV/EcoRI and filled in with
Klenow polymerase. The resulting plasmid, pOG9, was
found by DNA sequence analysis to have a 110 bp insert in
5    the H6 promoter between the NruI and EcoRV sites. To
remove these sequences, pOG9 was digested with NruI/EcoRV
and religated to yield the donor plasmid pC6FIPSH6II
which has the complete H6 promoter minus four base pairs
between the NruI and EcoRI sites which is not required
10   for early and late transcription. This plasmid consists
of the left arm of the C6 locus, the H6 promoter,
complete S gene ORF and the right arm of the C6 locus
(Figure 6 (SEQ ID NO:32)). A mutation in the stop codon
adds an additional nine amino acids to the C-terminus of
15   spike (Figure 7).

     This donor plasmid, pC6FIPSH6II, was used in *in vivo*
recombination (Piccini et al., 1987) with the ALVAC virus
vector to generate the recombinant virus vCP281.

     Immunoprecipitation analysis from a radiolabeled
20   lysate of CRFK cells infected with vCP281 using a FIP
spike specific monoclonal antibody designated 23F4.5
(Olsen et al., 1992) showed expression of a 220 kDa
polypeptide band. This was consistent with the expected
size of the S gene product. In addition, the band
25   comigrated with an immunoprecipitated band from FIPV
infected cells, consistent with proper glycosylation.
FACS analysis using the same monoclonal antibody showed
this expressed protein from vCP281 was localized in the
cytoplasm of the infected cell. However, inoculation of
30   monolayers of CRFK cells with vCP281 showed strong
fusigenic activity, indicating the protein was also on
the surface of these cells. No fusigenic activity was
observed in CRFK cells infected with the ALVAC parental
virus (control).

35        5.   Generation of an ALVAC Recombinant Expressing
               the FIPV Spike Glycoprotein Gene ORF Minus the
               Signal Sequence (vCP283B).

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The 57 bp signal sequence was removed from the N-
terminus of the S gene and replaced by an ATG by
inserting a 270 bp PCR fragment into pOG9 as follows.
The PCR fragment was amplified from pBSFIPS-A using
5   oligonucleotide primers RG759 (SEQ ID NO:33) (5'-
GCTATTTTCCATGGCTTCC-3') and RG760 (SEQ ID NO:34) (5'-
TCCGAGCTCGATATCCGTTAAGTTTGTATCGTAATGA-CAACAAATAATGAATGC-
3'). The fragment was digested with EcoRV/NcoI and
ligated into pOG9 digested with EcoRV/NcoI to yield
10  pOM12. pOM12 was digested with EcoRV/NruI and religated
to remove the 110 bp insert in the H6 promoter. The
resulting donor plasmid, pC6FIPSH6-SS, was confirmed by
DNA sequence analysis (Figure 7 (SEQ ID NO:35)).

This donor plasmid, pC6FIPSH6-SS, was used in *in*
15  *vivo* recombination (Piccini et al., 1987) with the ALVAC
virus vector to generate the recombinant virus vCP283B.

Immunoprecipitation analysis from a radiolabeled
lysate of CRFK cells infected with vCP283B using a cat
FIP-immune serum (#511) showed expression of a
20  polypeptide band of about 145±10 kDa. This was
consistent with the predicted size of a non-glycosylated
S gene product. Immunofluorescence analysis using the
same polyclonal serum showed this expressed protein was
localized in the cytoplasm of vCP283B infected CEF cells.
25  No fusigenic activity was observed in CRFK cells.

6.   Generation of an ALVAC Recombinant Expressing
the C-terminal Section of the FIPV Spike
Glycoprotein Gene ORF (vCP315).

30  The C-terminal 1749 bp of the S gene (terminal 582
aa out of 1452 aa total) was linked to the H6 promoter as
follows. pOG9 was digested with NruI/BstEII and a 6.2 kb
fragment isolated. This fragment contains the 1749 bp C-
terminal portion of the S gene. A fragment containing
35  the 3' end of the H6 promoter coupled to an ATG codon
flanked by a BstEII site was generated by annealing
oligonucleotides JP226 (SEQ ID NO:36) (5'-
CATTAGCATGATATCCGTTAAGTTTGTATCGT-AATGGGTAACCCTGAGTAGCAT-
3') and JP227 (SEQ ID NO:37) (5'-

ATGCTACTCAGGGTTACCCATTACGATACAAACTTAACGGATATCATGCTAATG-
3') and digesting with NruI/BstEII.  This fragment was
ligated into the 6.2 kb pOG9 fragment (see 4 above) to
yield the donor plasmid pC6FIPSH6-C, which was confirmed
5   by DNA sequence analysis (Figure 8 (SEQ ID NO:38)).

        This donor plasmid, pC6FIPSH6-C, was used in *in vivo*
recombination (Piccini et al., 1987) with the ALVAC virus
vector  to generate the recombinant virus vCP315.

        Western blot analysis from a lysate of CRFK cells
10  infected with vCP315 using a cat FIP-immune serum (#511)
showed expression of a 56 kDa polypeptide band.  This was
slightly smaller than the  predicted size of the
truncated, non-glycosylated S gene product (64 kDa).
Immunofluorescence analysis using the same polyclonal
15  serum showed a weak detection of the protein localized in
the cytoplasm of vCP315 infected CEF cells.  No fusigenic
activity was observed in CRFK cells.

**EXAMPLE 2   -   GENERATION OF C3, C5 AND C6 INSERTION
                  PLASMIDS**

20
        Generation of C3 insertion plasmid pSPCP3LA.

        An 8.5 kb canarypox BglII fragment was cloned into
the BamI site of pBluescript SK+ (Stratagene, La Jolla,
CA) to yield pWW5.  Nucleotide sequence analysis of this
25  fragment revealed an open reading frame designated C3
initiated at position 1458 and terminated at position
2897 in the sequence presented in Figure 9 (SEQ ID
NO:39).  In order to delete the entire C3 open reading
frame (ORF), PCR primers were designed to amplify a 5'
30  and a 3' fragment relative to the C3 ORF. Oligonucleotide
primers RG277 (SEQ ID NO:40) (5'-CAGTTG-
GTACCACTGGTATTTTATTTCAG-3') and RG278 (SEQ ID NO:41) (5'-
TATCTGAATTCCTGCAGCCCGGGTTTTTATAGCTAATTAGTCAAATG-
TGAGTTAATATTAG-3') were used to amplify the 5' fragment
35  from pWW5 and oligonucleotide primers RG279 (SEQ ID
NO:42)
        (5'TCGCTGAATTCGATATCAAGCTTATCGATTTTTATGACTAGTTAATCAAATAAA
AA-GCATACAAGC-3') were used to amplify the 3' fragment
from pWW5.  The 5' fragment was digested with

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Asp718/EcoRI and the 3' fragment digested with
EcoRI/SacI. The 5' and 3' arms were then ligated into
pBluescript SK+ digested with Asp718/SacI to yield pC3I.
This plasmid contains the C3 insertion locus with the C3
5   ORF deleted and replaced with a multiple cloning site
flanked by vaccinia early transcriptional and
translational termination signal. pC3I was confirmed by
DNA sequence analysis.

The flanking arms of pC3I were lengthened as
10  follows. A 908 bp fragment upstream of the C3 locus was
obtained by digestion of pWW5 with NsiI and SspI. A 604
bp PCR fragment was amplified from pWW5 using
oligonucleotide primers CP16 (SEQ ID NO:43)(5'-
TCCGGTACCGCGGCCGCAGATATTTGTTAGCTTCTGC-3') and CP17 (SEQ
15  ID NO:44) (5'-TCGCTCGAGTAGGATACCTACCTACTACCTA-CG-3'),
digested with Asp718/XhoI and ligated into pIBI25
(International Biotechnologies, Inc., New haven, CT) to
yield pSPC3LA. pSPC3LA was digested within pIBI25 with
EcORV and within the insert (canarypox DNA) with NsiI and
20  ligated to the 908 bp Nsi/SspI fragment generating
pSPCPLAX which contains 1444 bp of canarypox DNA upstream
of the C3 locus. A 2178 bp BglII/StyI fragment of
canarypox DNA was isolated from pXX4 (which contains a
6.5 kb NsiI fragment of canarypox DNA cloned into the
25  PstI site of pBluescript SK+). A 279 bp PCR fragment was
amplified from pXX4 using oligonucleotide primers CP19
(SEQ ID NO:45) (5'-TCGCTCGAGCTTTCTTGACAATAACATAG-3') and
CP20 (SEQ ID NO:46) (5'-TAGGAGCTCTTTATACTACTGGGTTACAAC-
3'), digested with XhoI/SacI and ligated into pIBI25
30  digested with SacI/XhoI to yield pSPC3RA.

To add additional unique sites to the multiple
cloning site (MCS) in pC3I, pC3I was digested with
EcoRI/ClaI (in the MCS) and ligated to kinased and
annealed oligonucleotides CP12 (SEQ ID NO:47) (5'-
35  AATTCCTCGAGGGATCC-3') and (SEQ ID NO:48) (5'-
CGGGATCCCTCG-AGG-3') (containing an EcoRI sticky end,
XhoI site, BamHI site and a sticky end compatible with
ClaI) to yield pSPCP3S. pSPCP3S was digested within the

canarypox sequences downstream of the C3 locus with StyI
and SacI (from pBluescript SK+)and ligated to a 261 bp
BglII/SacI fragment from pSPC3RA and the 2178 bp
BglII/StyI fragment from pXX4 generating pCPRAL
5      containing 2572 bp of canarypox sequences downstream of
the C3 locus. pSPCP3S was digested within the canarypox
sequences upstream of the C3 locus with Asp718 (in
pBluescript SK+) and AccI and ligated to a 1436 bp
Asp718/AccI fragment from pSPCPLAX generating pCPLAI
10     containing 1457 bp of canarypox DNA upstream of the C3
locus. pCPLAI was digested within the canarypox
sequences downstream of the C3 locus with StyI and SacI
(in pBluescript SK+) and ligated to a 2438 bp StyI/SacI
fragment from pCPRAL generating plasmid pSPCP3LA. The
15     left arm of pSPCP3LA was shortened by about 500 bp as
follows. pSPCP3LA was digested with NotI/NsiI and a
6433 bp fragment was isolated. Oligonucleotides CP34
(SEQ ID NO:49) (5'-GGCCGCGTCGACATGCA-3') and CP35 (SEQ ID
NO:50) (5'-TGTCGACGC-3') were annealed and ligated into
20     this fragment to yield pSPCP3LSA. This is the C3
insertion plasmid which consists of 939 bp of canarypox
DNA upstream of the C3 locus, stop codons in six reading
frames, early transcriptional termination signal, an MCS,
early transcriptional termination signal, stop codons in
25     six reading frames and 2572 bp of canarypox DNA
downstream of the C3 locus.

Generation of C5 insertion plasmid pNC5LSP-5.

A genomic library of canarypox DNA was constructed
in the cosmid vector pVK102 (Knauf and Nester, 1982)
30     probed with pRW764.5 (a pUC9 based plasmid containing an
880 bp canarypox PvuII fragment which includes the C5
ORF) and a cosmid clone containing a 29 kb insert was
identified (pHCOS1). A 3.3 kb ClaI fragment from pHCOS1
containing the C5 region was identified. The C5 ORF is
35     initiated at position 1537 and terminated at position
1857 in the sequence shown in Figure 10 (SEQ ID NO:51).

The C5 insertion vector was constructed in two
steps. The 1535 bp upstream sequence was generated by

PCR amplification from purified genomic canarypox DNA
using oligonucleotide primers C5A (SEQ ID NO:52) (5'-
ATCATCGAATTCTGAATGTTAAATGTTATACTTTG-3') and C5B (SEQ ID
NO:53) (5'-GGGGGTACCTTTGAGAGTACCACTTCAG-3'). This

5    fragment was digested with EcoRI and ligated into pUC8
digested with EcoRI/SmaI to yield pC5LAB. The 404 bp arm
was generated by PCR amplification using oligonucleotides
C5C (SEQ ID NO:54) (5'-
GGGTCTAGAGCGGCCGCTTATAAAGATCTAAAATGCATAATTTC-3') and C5DA

10   (SEQ ID NO:55) (5'-ATCATCCTGCAGGTATTCTAAACTAGGAATAGATG-
3'). This fragment was digested with PstI and cloned
into SmaI/PstI digested pC5LAB to yield pC5L. pC5L was
digested within the MCS with Asp718/NotI and ligated to
kinased and annealed oligonucleotides CP26 (SEQ ID NO:56)

15   (5'-
GTACGTGACTAATTAGCTATAAAAAGGATCCGGTACCCTCGAGTCTAGAATCGATCC-
CGGGTTTTTATGACTAGTTAATCAC-3') and CP27 (SEQ ID NO:57)
(5'-
GGCCGTGATTAACTAGTCATAAAAACCCGGGATCGATTCTAGACTCGAGGGTACCGG-

20   ATCCTTTTTATAGCTAATTAGTCAC-3') to yield pC5LSP. This
plasmid was digested with EcoRI, ligated with kinased
and self-annealed oligonucleotide CP29 (SEQ ID NO:58)
(5'-AATTGCGGCCGC-3') and digested with NotI. The
linearized plasmid was purified and self-ligated to

25   generate pNC5LSP-5. This C5 insertion plasmid contains
1535 bp of canarypox DNA upstream of the C5 ORF,
translation stop codons in six reading frames, vaccinia
early transcription termination signal, an MCS with
BamHI, KpnI, XhoI, ClaI and SmaI restriction sites,

30   vaccinia early termination signal, translation stop
codons in six reading frames and 404 bp of downstream
canarypox sequence (31 bp of C5 coding sequence and 373
bp of downstream canarypox sequence).

<u>Generation of C6 insertion plasmid pC6L</u>.

35   Figure 11 (SEQ ID NO:59) is the sequence of a 3.7 kb
segment of canarypox DNA. Analysis of the sequence
revealed an ORF designated C6L initiated at position 377
and terminated at position 2254. The following describes

a C6 insertion plasmid constructed by deleting the C6 ORF
and replacing it with an MCS flanked by transcriptional
and translational termination signals. A 380 bp PCR
fragment was amplified from genomic canarypox DNA using
5    oligonucleotide primers C6A1 (SEQ ID NO:60) (5'-
ATCATCGAG-CTCGCGGCCGCCTATCAAAAGTCTTAATGAGTT-3') and C6B1
(SEQ ID NO:61)
(5'GAATTCCTCGAGCTGCAGCCCGGGTTTTTATAGCTAATTAGTCATTTT-
TTCGTAAGTAAGTATTTTTATTTAA-3'). A 1155 bp PCR fragment
10   was amplified from genomic canarypox DNA using
oligonucleotide primers C6C1 (SEQ ID NO:62) (5'-
CCCGGGCTGCAGCTCGAGGAATTCTT-
TTTATTGATTAACTAGTCAAATGAGTATATATAATTGAAAAAGTAA-3') and
C6D1 (SEQ ID NO:63) (5'-
15   GATGATGGTACCTTCATAAATACAAGTTTGATTAAACTT-AAGTTG-3'). The
380 bp and 1155 bp fragments were fused together by
adding them together as template and amplifying a 1613 bp
PCR fragment using oligonucleotide primers C6A1 (SEQ ID
NO:49) and C6D1 (SEQ ID NO:52). This fragment was
20   digested with SacI/KpnI and ligated into pBluescript SK+
digested with SacI/KpnI. The resulting plasmid, pC6L was
confirmed by DNA sequence analysis. It consists of 370
bp of canarypox DNA upstream of C6, vaccinia early
termination signal, translation stop codons in six
25   reading frames, an MCS containing SmaI, PstI, XhoI and
EcoRI sites, vaccinia early termination signal,
translation stop codons in six reading frames and 1156 bp
of downstream canary pox sequence.

pJCA070 was derived from pC6L by ligating a cassette
30   containing the vaccinia H6 promoter coupled to another
foreign gene into the SmaI/EcoRI sites of pC6L. Cutting
pJCA070 with EcoRV/EcoRI excises the foreign gene and the
5' end of the H6 promoter.

**EXAMPLE 3   -   EFFICACY TRIALS WITH ALVAC-BASED FELINE**
35                  **INFECTIOUS PERITONITIS VIRUS RECOMBINANTS**

Trial 1   Safety, antigenicity and efficacy trial
          with vCP261A(N), vCP262 (M) and
          vCP282(M+N).
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Twenty five specific pathogen-free (SPF) 10-12 week
old cats from Harlan Sprague Dawley, Inc. were randomly
divided into five groups (5 cats/group). Groups were
vaccinated subcutaneously (neck area) twice (day 0 and
5    day 21) with $10^7$ $TCID_{50}$/dose with either vCP261, vCP262,
vCP282 or vCP261A + vCP262. Five cats in one group were
not vaccinated and served as challenge controls. At day
35, all cats were challenged orally with $10^{3.5}$ $TCID_{50}$ per
cat with a virulent FIP virus (strain 1146). The cats
10   were observed daily for 33 days post challenge to monitor
mortality and visible manifestations of FIP virus
infection. At day 33, all surviving cats were necropsied
and examined for FIP pathology. The non-effusive form was
detected by isolation of FIP virus from the intestinal
15   tract and identification by virus-neutralization tests.
Cats with the effusive form had a thick yellow fluid in
the peritoneal cavity, white edematous fluid in the
pleural cavity and lesions on the intestine, spleen and
liver. Some infected cats showed ocular involvement with
20   conjunctivitis, blepharospasm and opalesent retina.

None of the vaccinated cats showed any adverse local
or systemic postvaccination reactions. All five
nonvaccinated cats either died with FIP signs or when
necropsied had FIP signs, thus validating the challenge
25   dose. Dead and dying cats displayed signs of both
effusive and non-effusive forms of FIP. The results from
the ALVAC-FIP recombinant vaccinated cats is presented in
Table 1. None of these cats developed virus neutralizing
antibody prior to challenge on day 35. All cats had a
30   febrile response following challenge. All vaccinated
groups showed partial protection with the best protection
in the vCP262 and vCP282 vaccinated groups, each having
3/5 cats with no FIP mortality or signs. Thus, it
appears from this study that the ALVAC-FIP matrix
35   recombinants provided the best overall protection.

            Trial 2    Safety, antigenicity and efficacy trial
                       with vCP262 (M) in comparison with
                       PRIMUCELL.

44

Twenty three SPF cats aged 10-12 weeks from Hill
Grove, Great Britain were used in this trial. Ten cats
were vaccinated subcutaneously with vCP262 at a dose of
$10^8$ pfu on days 0 and 21. Five cats received a
5    commercially available FIP vaccine (PRIMUCELL, Smithkline
Beecham) which was given as recommended by the
manufacturer (2 doses, 21 days apart, intranasal, $10^{4.8}$
$TCID^{50}$ per dose). Eight cats were non-vaccinated and
served as challenge controls. On day 35, all cats were
10   challenged with a virulent FIP virus (strain 79-1146) at
a dose of 320 $DECP_{50}$ given intranasally. Surviving cats
were rechallenged on day 84 and those surviving were
necropsied on day 104 and examined for FIP pathology.

None of the vaccinated cats showed any adverse local
15   or systemic postvaccination reactions. Within the
control group, four of the cats either died or had FIP
pathology when necropsied. The remaining four controls
(housed in a separate unit from the other controls)
survived both challenges and appeared to be protected.
20   They all showed significant increase in serum
neutralizing antibodies to FIP following challenge, thus
indicating exposure to the virus. Whether this indicates
technical problems with the challenge protocol or a
natural protection is unknown.

25       Serological analysis showed no significant viral
neutralizing antibody titers to FIP in cats receiving two
inoculations of vCP262. In contrast, significant titers
were observed after one inoculation of PRIMUCELL and
these titers were boosted after the second inoculation.
30   Cats in both groups showed high titers following
challenge.

The mortality data results for the vaccinated cats
is presented in Table 2. In the vCP262 group, 8/10 cats
(80%) survived the first challenge, while 6/10 (60%)
35   survived both challenges (60%). In contrast, in the
PRIMUCELL group, only 1/5 cats survived the first
challenge. The surviving cat also survived the second
challenge. It is important to note that 3 of the 4 dead

PRIMUCELL vaccinated cats died on or before day 11 which indicates an enhancement of the normal progression of the disease.  No enhancement was observed with vCP262 vaccinated cats.  Thus, compared to PRIMUCELL, vCP262

5  provides greater protection with no enhancement of the disease.

Trial 3    Safety, antigenicity and efficacy trial with vCP262 (M) in combination with the spike recombinants (vCP281(S1), vCP283B(S2) and vCP315(S3)).

10

Thirty six 9 week old SPF cats were received from Harlan Sprague Dawley, Inc. and randomly divided into six groups (6 cats/group).  Groups received two subcutaneous

15  inoculations (dose of about $10^7$ $TCID_{50}$ for each recombinant at day 0 and day 21,) with the following recombinants: 1) vCP262 (matrix), 2) vCP262 plus vCP281 (S1 spike - complete), 3) vCP262 plus vCP283B (S2 spike - minus signal sequence) and 4) vCP262 plus vCP315 (S3

20  spike - C-terminal section).  One group was vaccinated intranasally with a commercially available FIP vaccine (PRIMUCELL, Pfizer Animal Health) as recommended by the manufacturer (2 doses, day 0 and day 21).  One group was not vaccinated and served as challenge controls.  Fifteen

25  days following the second vaccination (day 36), all cats were challenged orally with $10^{3.5}$ $TCID_{50}$ per cat with a virulent FIP virus (NVSL FIP-1146, 89-5-1).  The cats were monitored for weight, temperature, serologic response and mortality for 35 days post challenge.

30  Necropsy was performed on the majority of dead cats to look for FIP signs and FIPV virus was isolated from two cats to confirm infection.

None of the cats vaccinated with ALVAC recombinants showed any adverse local or systemic postvaccination

35  reactions.  All cats vaccinated with PRIMUCELL had virus neutralizing titers.  In the recombinant groups, only cats in the group receiving matrix plus complete spike had virus neutralizing titers (3/6 after the second vaccination).

46

The mortality data is presented in table 3. Necropsied cats showed signs of both the effusive (majority) and non-effusive forms of the disease. One cat had FIP induced encephalitis (control group). The

5   lowest mortality (33%) was observed in the group vaccinated with vCP262 (matrix) alone. Groups receiving vCP262 plus any of the spike recombinants showed little, if any protection. The PRIMUCELL vaccinated group showed a mortality of 66.7%. Antibody induced enhancement

10  (early death) was observed in both the PRIMUCELL and vCP281 (S1 - complete spike) groups. Five out of six (83.3%) of the control nonvaccinated cats died from FIP infection which validated the challenge.

Fever and weight loss are indicators of FIP disease.

15  There was relative postchallenge weight loss in all the groups. However the vCP262 vaccinated group showed only a slight weight loss as compared to PRIMUCELL and the control groups. Chronic fever was observed in all cats, however the group that was vaccinated with vCP262

20  exhibited consistently lower temperatures that the other groups.

From this study it was concluded that vCP262 provided protection (67.7%) against a severe FIP challenge. In addition, cats vaccinated with this

25  recombinant showed a lower febrile response and less weight loss following challenge. The other FIP recombinants (vCP281, vCP283B, and vCP315) as well as PRIMUCELL provided poor protection and even enhancement of mortality (PRIMUCELL, vCP281).

**TABLE 1**     Results of FIP Efficacy Trial with ALVAC Matrix
           & Nucleocapsid Recombinants

| Groups | Virus Neutralizing Antibody Titer (GMAT)[1] | | Mortality | | Protection[3] |
|---|---|---|---|---|---|
| | Day 35 | Day 63 | Alive[2] | Dead | |
| Control | <2 | >14,190 | 2(2FIP+) | 3 | 0/5 (0%) |
| vCP261A (N) | <2 | 446 | 2(1FIP+) | 3 | 1/4 (20%) |
| vCP262 (M) | <2 | >11,585 | 4(1FIP+) | 1 | 3/5 (60%) |
| vCP282 (M+N) | <2 | >16,384 | 4(1FIP+) | 1 | 3/5 (60%) |
| vCP261A (N) + vCP262 (M) | <2 | >16,384 | 3(1FIP+) | 2 | 2/5 (40%) |

1.    Titers expressed as reciprocal of final serum
      dilution.

2.    Numbers in parenthesis represent cats with FIP signs
      at necropsy.

3.    No mortality or FIP signs.

48

**TABLE 2**    Results of Efficacy Trial Comparing ALVAC
Matrix Recombinant with PRIMUCELL

| Groups | Number of Cats | Mortality | | Protection |
|---|---|---|---|---|
| | | 1st Challenge Day 35 | 2nd Challenge[1] Day 84 | |
| Control | 8 | 3 | 1 | 4/8 (50%) |
| vCP262 (M) | 10 | 2 | 2 | 6/10 (60%) |
| PRIMUCELL | 5 | 4[2] | 0 | 1/5 (20%) |

1.    Includes cats necropsied with FIP pathology at day
104.

2.    Three of these cats died on or before day 11
indicating enhancement.

**TABLE 3**   Mortality Data Comparing ALVAC-based Matrix and Spike Recombinants with PRIMUCELL.

| Group | Mortality | Enhancement[1] |
|---|---|---|
| vCP262 (M) | 2/6 (33%) | NO |
| vCP262 (M) + vCP281 (S1) | 6/6 (100%) | YES |
| vCP262 (M) + vCP283 (S2) | 5/6 (83.3%) | NO |
| vCP262 (M) + vCP315 (S3) | 5/6 (83.3%) | NO |
| PRIMUCELL | 4/6 (66.7%) | YES |
| Control | 5/6 (83.3%) | NO |

1.   Death on or prior to day 15 post challenge.

## EXAMPLE 4   -   GENERATION OF NYVAC-BASED FIPV
## RECOMBINANTS

Using insertion loci and promoters as in USSN
105,483, incorporated herein by reference, such as by
5   modifying plasmid pRW842 for insertion of rabies
glycoprotein G gene into TK deletion locus (used for
generation of vP879), e.g., by excising out of pRW842 the
rabies DNA and inserting therefor the herein disclosed
FIPV DNA coding for M, N, and the three versions of S;
10  S1, S2, S3, or combinations thereof (for instance M and
N) and by then employing the resultant plasmids in
recombination with NYVAC, vP866, NYVAC-FIPV(M), (N), and
the three versions of (S); (S1), (S2), (S3), and (M + N)
recombinants are generated; and analysis confirms
15  expression.

Having thus described in detail preferred
embodiments of the present invention, it is to be
understood that the invention defined by the appended
claims is not to be limited by particular details set
20  forth in the above description as many apparent
variations thereof are possible without departing from
the spirit or scope thereof.

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**WHAT IS CLAIMED IS:**

1.    A recombinant poxvirus containing therein
DNA from feline infectious peritonitis virus in a non-
essential region of the poxvirus genome wherein the
5  poxvirus is

(i) a vaccinia virus wherein J2R, B13R +
B14R, A26L, A56R, C7L-K1L and I4L are deleted from the
virus, or a thymidine kinase gene, a hemorrhagic region,
an A type inclusion body region, a hemagglutinin gene, a
10  host range region, and a large subunit, ribonucleotide
reductase are deleted from the virus; or, the poxvirus is

(ii) canarypox which was attenuated
through more than 200 serial passages on chick embryo
fibroblasts, a master seed therefrom was subjected to
15  four successive plaque purifications under agar, from
which a plaque clone was amplified through five
additional passages.

2.    The recombinant of claim 1 wherein the
poxvirus is the canarypox virus.

20        3.    The recombinant of claim 2 which is
vCP262, vCP261A, vCP282, vCP281, vCP283B, vCP315.

4.    The recombinant of claim 1 wherein the
feline infectious peritonitis virus DNA encodes M, N, and
the three versions of S; S1, S2, S3, or combinations
25  thereof.

5.    The recombinant of claim 4 wherein the DNA
encodes M.

6.    The recombinant of claim 4 wherein the DNA
encodes N.

30        7.    The recombinant of claim 4 wherein the DNA
encodes S.

8.    The recombinant of claim 4 wherein the DNA
encodes S1.

9.    The recombinant of claim 4 wherein the DNA
35  encodes S2.

10.    The recombinant of claim 4 wherein the DNA
encodes S3.

11. The recombinant of claim 4 wherein the DNA encodes M+N.

12. The recombinant of claim 1 wherein the poxvirus is the vaccinia virus.

13. The recombinant of claim 3 which is vCP262.

14. An immunological composition comprising a recombinant as claimed in claim 1, 2, 3, 11, 12 or 13, and a carrier.

15. A method for inducing an immunological response in a host comprising administering a recombinant as claimed in anyone of claims 1, 2, 3, 11, 12 or 13.

16. A method for inducing an immunlogical response comprising administering a composition as claimed in claim 14.

17. A method for expressing a gene product *in vitro* comprising infecting a cell culture with a recombinant as claimed in claim 1, 2, 3, 11, 12 or 13.

Figure 1

```
  1 ATGAAGTACA TTTTGCTAAT ACTCGCGTGC ATAATTGCAT GCGTTTATGG TGAACGCTAC
 61 TGTGCCATGC AAGACAGTGG CTTGCAGTGT ATTAATGGCA CAAATTCAAG ATGTCAAACC
121 TGCTTTGAAC GTGGTGATCT TATTTGGCAT CTTGCTAACT GGAACTTCAG CTGGTCTGTA
181 ATATTGATTG TTTTTATAAC AGTGTTACAA TATGGCAGAC CACAATTTAG CTGGCTCGTT
241 TATGGCATTA AAATGCTGAT CATGTGGCTA TTATGGCCTA TTGTTCTAGC GCTTACGATT
301 TTTAATGCAT ACTCTGAGTA CCAAGTTTCC AGATATGTAA TGTTCGGCTT TAGTGTTGCA
361 GGTGCAGTTG TAACGTTTGC ACTTTGGATG ATGTATTTTG TGAGATCTGT TCAGCTATAT
421 AGAAGAACCA AATCATGGTG GTCTTTTAAT CCTGAGACTA ATGCAATTCT TTGTGTTAAT
481 GCATTGGGTA GAAGTTATGT GCTTCCCTTA GATGGTACTC CTACAGGTGT TACCCTTACT
541 CTACTTTCAG GAAATCTATA TGCTGAAGGT TTCAAAATGG CTGGTGGTTT AACCATCGAG
601 CATTTGCCTA AATACGTCAT GATTGCTACA CCTAGTAGAA CCATCGTTTA TACATTAGTT
661 GGAAAACAAT TAAAAGCAAC TACTGCCACA GGATGGGCTT ACTACGTAAA ATCTAAAGCT
721 GGTGATTACT CAACAGAAGC ACGTACTGAC AATTTGAGTG AACATGAAAA ATTATTACAT
781 ATGGTGTAA
```

Figure 2

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   1 GAATTGCGGC CGCTGAATGT TAAATGTTAT ACTTTGGATG AAGCTATAAA TATGCATTGG
  61 AAAAATAATC CATTTAAAGA AAGGATTCAA ATACTACAAA ACCTAAGCGA TAATATGTTA
 121 ACTAAGCTTA TTCTTAACGA CGCTTTAAAT ATACACAAAT AAACATAATT TTTGTATAAC
 181 CTAACAAATA ACTAAAACAT AAAAATAATA AAAGGAAATG TAATATCGTA ATTATTTTAC
 241 TCAGGAATGG GGTTAAATAT TTATATCACG TGTATATCTA TACTGTTATC GTATACTCTT
 301 TACAATTACT ATTACGAATA TGCAAGAGAT AATAAGATTA CGTATTTAAG AGAATCTTGT
 361 CATGATAATT GGGTACGACA TAGTGATAAA TGCTATTTCG CATCGTTACA TAAAGTCAGT
 421 TGGAAAGATG GATTTGACAG ATGTAACTTA ATAGGTGCAA AAATGTTAAA TAACAGCATT
 481 CTATCGGAAG ATAGGATACC AGTTATATTA TACAAAAATC ACTGGTTGGA TAAAACAGAT
 541 TCTGCAATAT TCGTAAAAGA TGAAGATTAC TGCGAATTTG TAAACTATGA CAATAAAAAG
 601 CCATTTATCT CAACGACATC GTGTAATTCT TCCATGTTTT ATGTATGTGT TTCAGATATT
 661 ATGAGATTAC TATAAACTTT TTGTATACTT ATATTCCGTA AACTATATTA ATCATGAAGA
 721 AAATGAAAAA GTATAGAAGC TGTTCACGAG CGGTTGTTGA AAACAACAAA ATTATACATT
 781 CAAGATGGCT TACATATACG TCTGTGAGGC TATCATGGAT AATGACAATG CATCTCTAAA
 841 TAGGTTTTTG GACAATGGAT TCGACCCTAA CACGGAATAT GGTACTCTAC AATCTCCTCT
 901 TGAAATGGCT GTAATGTTCA AGAATACCGA GGCTATAAAA ATCTTGATGA GGTATGGAGC
 961 TAAACCTGTA GTTACTGAAT GCACAACTTC TTGTCTGCAT GATGCGGTGT TGAGAGACGA
1021 CTACAAAATA GTGAAGATC TGTTGAAGAA TAACTATGTA AACAATGTTC TTTACAGCGG
1081 AGGCTTTACT CCTTTGTGTT TGGCAGCTTA CCTTAACAAA GTTAATTTGG TTAAACTTCT
1141 ATTGGCTCAT TCGGCGGATG TAGATATTTC AAACACGGAT CGGTTAACTC CTCTACATAT
1201 AGCCGTATCA AATAAAAATT TAACAATGGT TAAACTTCTA TTGAACAAAG GTGCTGATAC
1261 TGACTTGCTG GATAACATGG GACGTACTCC TTTAATGATC GCTGTACAAT CTGGAAATAT
1321 TGAAATATGT AGCACACTAC TTAAAAAAAA TAAAATGTCC AGAACTGGGA AAAATTGATC
1381 TTGCCAGCTG TAATTCATGG TAGAAAAGAA GTGCTCAGGC TACTTTTCAA CAAAGGAGCA
1441 GATGTAAACT ACATCTTTGA AAGAAATGGA AAATCATATA CTGTTTTGGA ATTGATTAAA
1501 GAAAGTTACT CTGAGACACA AAAGAGGTAG CTGAAGTGGT ACTCTCAAAG GTACGTGACT
1561 AATTAGCTAT AAAAAGGATC CGGTACCCTC GAGTCTAGAA TCGATCCCGT ACCGTTTAGT
1621 TACACCATAT GTAATAATTT TTCATGTTCA CTCAAATTGT CAGTACGTGC TTCTGTTGAG
1681 TAATCACCAG CTTTAGATTT TACGTAGTAA GCCCATCCTG TGGCAGTAGT TGCTTTTAAT
1741 TGTTTTCCAA CTAATGTATA AACGATGGTT CTACTAGGTG TAGCAATCAT GACGTATTTA
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Figure 2 (cont'd.)

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1801 GGCAAATGCT CGATGGTTAA ACCACCAGCC ATTTTGAAAC CTTCAGCATA TAGATTTCCT
1861 GAAAGTAGAG TAAGGGTAAC ACCTGTAGGA GTACCATCTA AGGGAAGCAC ATAACTTCTA
1921 CCCAATGCAT TAACACAAAG AATTGCATTA GTCTCAGGAT TAAAAGACCA CCATGATTTG
1981 GTTCTTCTAT ATAGCTGAAC AGATCTCACA AAATACATCA TCCAAAGTGC AAACGTTACA
2041 ACTGCACCTG CAACACTAAA GCCGAACATT ACATATCTGG AAACTTGGTA CTCAGAGTAT
2101 GCATTAAAAA TCGTAAGCGC TAGAACAATA GGCCATAATA GCCACATGAT CAGCATTTTA
2161 ATGCCATAAA CGAGCCAGCT AAATTGTGGT CTGCCATATT GTAACACTGT TATAAATACT
2221 ATCAATATTA CAGACCAGCT GAAGTTCCAG TTAGCAAGAT GCCAAATAAG ATCACCACGT
2281 TCAAAGCAGG TTTGACATCT TGAATTTGTG CCATTAATAC ACTGCAAGCC ACTGTCTTGC
2341 ATGGCACAGT AGCGTTCACC ATAAACGCAT GCAATTATGC ACGCGAGTAT TAGCAAAATG
2401 TACTTCATTT TATATTGTAA TTATATATTT TCAATTTTGA AATCCCAAAA TATTATCATA
2461 TTCTTCCCAA TAAAGAGCTC TAATTAATTA ACGAGCAGAT AGTCTCGTTC TCGCCCTGCC
2521 TGATGACTAA TTAATTAACC CGGGAAGCTG GGTTTTTATG ACTAGTTAAT CACGGCCGCT
2581 TATAAAGATC TAAAATGCAT AATTTCTAAA TAATGAAAAA AAGTACATCA TGAGCAACGC
2641 GTTAGTATAT TTTACAATGG AGATTAACGC TCTATACCGT TCTATGTTTA TTGATTCAGA
2701 TGATGTTTTA GAAAAGAAAG TTATTGAATA TGAAAACTTT AATGAAGATG AAGATGACGA
2761 CGATGATTAT TGTTGTAAAT CTGTTTTAGA TGAAGAAGAT GACGCGCTAA AGTATACTAT
2821 GGTTACAAAG TATAAGTCTA TACTACTAAT GGCGACTTGT GCAAGAAGGT ATAGTATAGT
2881 GAAAATGTTG TTAGATTATG ATTATGAAAA ACCAAATAAA TCAGATCCAT ATCTAAAGGT
2941 ATCTCCTTTG CACATAATTT CATCTATTCC TAGTTTAGAA TACCTGCAG
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Figure 3

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   1 ATGGCCACAC AGGGACAACG CGTCAACTGG GGAGATGAAC CTTCCAAAAG ACGTGGTCGT
  61 TCTAACTCTC GTGGTCGGAA GAATAATGAT ATACCTTTGT CATTCTACAA CCCCATTACC
 121 CTCGAACAAG GATCTAAATT TTGGAATTTA TGTCCGAGAG ACCTTGTTCC CAAAGGAATA
 181 GGTAATAAGG ATCAACAAAT TGGTTATTGG AATAGACAGA TTCGTTATCG TATTGTAAAA
 241 GGCCAGCGTA AGGAACTCGC TGAGAGGTGG TTCTTTTACT CTTAGGTAC AGGACCTCAT
 301 GCTGATGCTA AATTCAAAGA CAAGATTGAT GGAGTCTTCT GGGTTGCAAG GGATGGTGCC
 361 ATGAACAAGC CCACAACGCT TGGCACTCGT GGAACCAATA ACGAATCCAA ACCACTGAGA
 421 TTTGATGGTA AGATACCGCC ACAGTTTCAG CTTGAAGTGA ACCGTTCTAG GAACAATTCA
 481 AGGTCTGGTT CTCAGTCTAG ATCTGTTTCA AGAAACAGAT CTCAATCTAG AGGAAGACAC
 541 CATTCCAATA ACCAGAATAA TAATGTTGAG GATACAATTG TAGCCGTGCT TGAAAAATTA
 601 GGTGTTACTG ACAAACAAAG GTCACGTTCT AAACCTAGAG AACGTAGTGA TTCCAAACCT
 661 AGGGACACAA CACCTAAGAA TGCCAACAAA CACACCTGGA AGAAAACTGC AGGCAAGGGA
 721 GATGTGACAA CTTTCTATGG TGCTAGAAGT AGTTCAGCTA ACTTTGGTGA TAGTGATCTC
 781 GTTGCCAATG GTAACGCTGC CAAATGCTAC CCTCAGATAG CTGAATGTGT TCCATCAGTG
 841 TCTAGCATAA TCTTTGGCAG TCAATGGTCT GCTGAAGAAG CTGGTGATCA AGTGAAAGTC
 901 ACGCTCACTC ACACCTACTA CCTGCCAAAG GATGATGCCA AAACTAGTCA ATTCCTAGAA
 961 CAGATTGACG CTTACAAGCG ACCTTCTGAA GTGGCTAAGG ATCAGAGGCA AGAAGATCC
1021 CGTTCTAAGT CTGCTGATAA GAAGCCTGAG GAGTTGTCTG TAACTCTTGT GGAGGCATAC
1081 ACAGATGTGT TTGATGACAC ACAGGTTGAG ATGATTGATG AGGTTACGAA CTAA
```

Figure 4

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   1 GCGGCCGCGT CGACATGCAT TGTTAGTTCT GTAGATCAGT AACGTATAGC ATACGAGTAT

  61 AATTATCGTA GGTAGTAGGT ATCCTAAAAT AAATCTGATA CAGATAATAA CTTTGTAAAT

 121 CAATTCAGCA ATTTCTCTAT TATCATGATA ATGATTAATA CACAGCGTGT CGTTATTTTT

 181 TGTTACGATA GTATTTCTAA AGTAAAGAGC AGGAATCCCT AGTATAATAG AAATAATCCA

 241 TATGAAAAAT ATAGTAATGT ACATATTTCT AATGTTAACA TATTTATAGG TAAATCCAGG

 301 AAGGGTAATT TTTACATATC TATATACGCT TATTACAGTT ATTAAAAATA TACTTGCAAA

 361 CATGTTAGAA GTAAAAAAGA AAGAACTAAT TTTACAAAGT GCTTTACCAA AATGCCAATG

 421 GAAATTACTT AGTATGTATA TAATGTATAA AGGTATGAAT ATCACAAACA GCAAATCGGC

 481 TATTCCCAAG TTGAGAAACG GTATAATAGA TATATTTCTA GATACCATTA ATAACCTTAT

 541 AAGCTTGACG TTTCCTATAA TGCCTACTAA GAAAACTAGA AGATACATAC ATACTAACGC

 601 CATACGAGAG TAACTACTCA TCGTATAACT ACTGTTGCTA ACAGTGACAC TGATGTTATA

 661 ACTCATCTTT GATGTGGTAT AAATGTATAA TAACTATATT ACACTGGTAT TTTATTTCAG

 721 TTATATACTA TATAGTATTA AAAATTATAT TTGTATAATT ATATTATTAT ATTCAGTGTA

 781 GAAAGTAAAA TACTATAAAT ATGTATCTCT TATTTATAAC TTATTAGTAA AGTATGTACT

 841 ATTCAGTTAT ATTGTTTTAT AAAAGCTAAA TGCTACTAGA TTGATATAAA TGAATATGTA

 901 ATAAATTAGT AATGTAGTAT ACTAATATTA ACTCACATTT GACTAATTAG CTATAAAAAC

 961 CCGTACCTTA GTTCGTAACC TCATCAATCA TCTCAACCTG TGTGTCATCA AACACATCTG

1021 TGTATGCCTC CACAAGAGTT ACAGACAACT CCTCAGGCTT CTTATCAGCA GACTTAGAAC

1081 GGGATCTTCT TTGCCTCTGA TCCTTAGCCA CTTCAGAAGG TCGCTTGTAA GCGTCAATCT

1141 GTTCTAGGAA TTGACTAGTT TTGGCATCAT CCTTTGGCAG GTAGTAGGTG TGAGTGAGCG

1201 TGACTTTCAC TTGATCACCA GCTTCTTCAG CAGACCATTG ACTGCCAAAG ATTATGCTAG

1261 ACACTGATGG AACACATTCA GCTATCTGAG GGTAGCATTT GGCAGCGTTA CCATTGGCAA

1321 CGAGATCACT ATCACCAAAG TTAGCTGAAC TACTTCTAGC ACCATAGAAA GTTGTCACAT

1381 CTCCCTTGCC TGCAGTTTTC TTCCAGGTGT GTTTGTTGGC ATTCTTAGGT GTTGTGTCCC

1441 TAGGTTTGGA ATCACTACGT TCTCTAGGTT TAGAACGTGA CCTTTGTTTG TCAGTAACAC

1501 CTAATTTTTC AAGCACGGCT ACAATTGTAT CCTCAACATT ATTATTCTGG TTATTGGAAT

1561 GGTGTCTTCC TCTAGATTGA GATCTGTTTC TTGAAACAGA TCTAGACTGA GAACCAGACC

1621 TTGAATTGTT CCTAGAACGG TTCACTTCAA GCTGAAACTG TGGCGGTATC TTACCATCAA

1681 ATCTCAGTGG TTTGGATTCG TTATTGGTTC CACGAGTGCC AAGCGTTGTG GGCTTGTTCA

1741 TGGCACCATC CCTTGCAACC CAGAAGACTC CATCAATCTT GTCTTTGAAT TTAGCATCAG
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Figure 4 (cont'd.)

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1801 CATGAGGTCC TGTACCTAAG AAGTAAAAGA ACCACCTCTC AGCGAGTTCC TTACGCTGGC

1861 CTTTTACAAT ACGATAACGA ATCTGTCTAT TCCAATAACC AATTTGTTGA TCCTTATTAC

1921 CTATTCCTTT GGGAACAAGG TCTCTCGGAC ATAAATTCCA AAATTTAGAT CCTTGTTCGA

1981 GGGTAATGGG GTTGTAGAAT GACAAAGGTA TATCATTATT CTTCCGACCA CGAGAGTTAG

2041 AACGACCACG TCTTTTGGAA GGTTCATCTC CCCAGTTGAC GCGTTGTCCC TGTGTGGCCA

2101 TGATTAAACC TAAATAATTG TACTTTGTAA TATAATGATA TATATTTTCA CTTTATCTCA

2161 GAGCTCTAAT TAATTAACGA GCAGATAGTC TCGTTCTCGC CCTGCCTGAT GACTAATTAA

2221 TTAACCCGGG AAGCTGGGCT GCAGGAATTC CTCGAGGGAT CCCGATTTTT ATGACTAGTT

2281 AATCAAATAA AAAGCATACA AGCTATTGCT TCGCTATCGT TACAAAATGG CAGGAATTTT

2341 GTGTAAACTA AGCCACATAC TTGCCAATGA AAAAAATAGT AGAAAGGATA CTATTTTAAT

2401 GGGATTAGAT GTTAAGGTTC CTTGGGATTA TAGTAACTGG GCATCTGTTA ACTTTTACGA

2461 CGTTAGGTTA GATACTGATG TTACAGATTA TAATAATGTT ACAATAAAAT ACATGACAGG

2521 ATGTGATATT TTTCCTCATA TAACTCTTGG AATAGCAAAT ATGGATCAAT GTGATAGATT

2581 TGAAAATTTC AAAAAGCAAA TAACTGATCA AGATTTACAG ACTATTTCTA TAGTCTGTAA

2641 AGAAGAGATG TGTTTTCCTC AGAGTAACGC CTCTAAACAG TTGGGAGCGA AAGGATGCGC

2701 TGTAGTTATG AAACTGGAGG TATCTGATGA ACTTAGAGCC CTAAGAAATG TTCTGCTGAA

2761 TGCGGTACCC TGTTCGAAGG ACGTGTTTGG TGATATCACA GTAGATAATC CGTGGAATCC

2821 TCACATAACA GTAGGATATG TTAAGGAGGA CGATGTCGAA AACAAGAAAC GCCTAATGGA

2881 GTGCATGTCC AAGTTTAGGG GGCAAGAAAT ACAAGTTCTA GGATGGTATT AATAAGTATC

2941 TAAGTATTTG GTATAATTTA TTAAATAGTA TAATTATAAC AAATAATAAA TAACATGATA

3001 ACGGTTTTTA TTAGAATAAA ATAGAGATAA TATCATAATG ATATATAATA CTTCATTACC

3061 AGAAATGAGT AATGGAAGAC TTATAAATGA ACTGCATAAA GCTATAAGGT ATAGAGATAT

3121 AAATTTAGTA AGGTATATAC TTAAAAAATG CAAATACAAT AACGTAAATA TACTATCAAC

3181 GTCTTTGTAT TTAGCCGTAA GTATTTCTGA TATAGAAATG GTAAAATTAT TACTAGAACA

3241 CGGTGCCGAT ATTTTAAAAT GTAAAAATCC TCCTCTTCAT AAAGCTGCTA GTTTAGATAA

3301 TACAGAAATT GCTAAACTAC TAATAGATTC TGGCGCTGAC ATAGAACAGA TACATTCTGG

3361 AAATAGTCCG TTATATATTT CTGTATATAG AAACAATAAG TCATTAACTA GATATTTATT

3421 AAAAAAAGGT GTTAATTGTA ATAGATTCTT TCTAAATTAT TACGATGTAC TGTATGATAA

3481 GATATCTGAT GATATGTATA AAATATTTAT AGATTTTAAT ATTGATCTTA ATATACAAAC

3541 TAGAAATTTT GAAACTCCGT TACATTACGC TATAAAGTAT AAGAATATAG ATTTAATTAG
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Figure 4 (cont'd.)

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3601 GATATTGTTA GATAATAGTA TTAAAATAGA TAAAAGTTTA TTTTTGCATA AACAGTATCT
3661 CATAAAGGCA CTTAAAAATA ATTGTAGTTA CGATATAATA GCGTTACTTA TAAATCACGG
3721 AGTGCCTATA AACGAACAAG ATGATTTAGG TAAAACCCCA TTACATCATT CGGTAATTAA
3781 TAGAAGAAAA GATGTAACAG CACTTCTGTT AAATCTAGGA GCTGATATAA ACGTAATAGA
3841 TGACTGTATG GGCAGTCCCT TACATTACGC TGTTTCACGT AACGATATCG AAACAACAAA
3901 GACACTTTTA GAAAGAGGAT CTAATGTTAA TGTGGTTAAT AATCATATAG ATACCGTTCT
3961 AAATATAGCT GTTGCATCTA AAAACAAAAC TATAGTAAAC TTATTACTGA AGTACGGTAC
4021 TGATACAAAG TTGGTAGGAT TAGATAAACA TGTTATTCAC ATAGCTATAG AAATGAAAGA
4081 TATTAATATA CTGAATGCGA TCTTATTATA TGGTTGCTAT GTAAACGTCT ATAATCATAA
4141 AGGTTTCACT CCTCTATACA TGGCAGTTAG TTCTATGAAA ACAGAATTTG TTAAACTCTT
4201 ACTTGACCAC GGTGCTTACG TAAATGCTAA AGCTAAGTTA TCTGGAAATA CTCCTTTACA
4261 TAAAGCTATG TTATCTAATA GTTTTAATAA TATAAAATTA CTTTTATCTT ATAACGCCGA
4321 CTATAATTCT CTAAATAATC ACGGTAATAC GCCTCTAACT TGTGTTAGCT TTTTAGATGA
4381 CAAGATAGCT ATTATGATAA TATCTAAAAT GATGTTAGAA ATATCTAAAA ATCCTGAAAT
4441 AGCTAATTCA GAAGGTTTTA TAGTAAACAT GGAACATATA AACAGTAATA AAAGACTACT
4501 ATCTATAAAA GAATCATGCG AAAAAGAACT AGATGTTATA ACACATATAA AGTTAAATTC
4561 TATATATTCT TTTAATATCT TTCTTGACAA TAACATAGAT CTTATGGTAA AGTTCGTAAC
4621 TAATCCTAGA GTTAATAAGA TACCTGCATG TATACGTATA TATAGGGAAT TAATACGGAA
4681 AAATAAATCA TTAGCTTTTC ATAGACATCA GCTAATAGTT AAAGCTGTAA AAGAGAGTAA
4741 GAATCTAGGA ATAATAGGTA GGTTACCTAT AGATATCAAA CATATAATAA TGGAACTATT
4801 AAGTAATAAT GATTTACATT CTGTTATCAC CAGCTGTTGT AACCCAGTAG TATAAAG
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Figure 5

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   1 ATGATTGTGC TCGTAACTTG CCTCTTGTTG TTATGTTCAT ACCACACAGT TTTGAGTACA
  61 ACAAATAATG AATGCATACA AGTTAACGTA ACACAATTGG CTGGCAATGA AAACCTTATC
 121 AGAGATTTTC TGTTTAGTAA CTTTAAAGAA GAAGGAAGTG TAGTTGTTGG TGGTTATTAC
 181 CCTACAGAGG TGTGGTACAA CTGCTCTAGA ACAGCTCGAA CTACTGCCTT TCAGTATTTT
 241 AATAATATAC ATGCCTTTTA TTTTGTTATG GAAGCCATGG AAAATAGCAC TGGTAATGCA
 301 CGTGGTAAAC CATTATTATT TCATGTGCAT GGTGAGCCTG TTAGTGTTAT TATATCGGCT
 361 TATAGGGATG ATGTGCAACA AAGGCCCCTT TTAAAACATG GGTTAGTGTG CATAACTAAA
 421 AATCGCCATA TTAACTATGA ACAATTCACC TCCAACCAGT GGAATTCCAC ATGTACGGGT
 481 GCTGACAGAA AAATTCCTTT CTCTGTCATA CCCACGGACA ATGGAACAAA AATCTATGGT
 541 CTTGAGTGGA ATGATGACTT TGTTACAGCT TATATTAGTG GTCGTTCTTA TCACTTGAAC
 601 ATCAATACTA ATTGGTTTAA CAATGTCACA CTTTTGTATT CACGCTCAAG CACTGCTACC
 661 TGGGAATACA GTGCTGCATA TGCTTACCAA GGTGTTTCTA ACTTCACTTA TTACAAGTTA
 721 AATAACACCA ATGGTCTAAA AACCTATGAA TTATGTGAAG ATTATGAACA TTGCACTGGC
 781 TATGCTACCA ATGTATTTGC TCCGACATCA GGTGGTTACA TACCTGATGG ATTTAGTTTT
 841 AACAATTGGT TCTTGCTTAC AAATAGTTCC ACTTTTGTTA GTGGCAGGTT TGTAACAAAT
 901 CAACCATTAT TGATTAATTG CTTGTGGCCA GTGCCCAGTT TTGGTGTAGC AGCACAAGAA
 961 TTTTGTTTTG AAGGTGCACA GTTTAGCCAA TGTAATGGTG TGTCTTTAAA TAACACAGTG
1021 GATGTTATTA GATTCAACCT TAATTTCACT GCAGATGTAC AATCTGGTAT GGGTGCTACA
1081 GTATTTTCAC TGAATACAAC AGGTGGTGTC ATTCTTGAAA TTTCATGTTA TAGTGACACA
1141 GTGAGTGAGT CTAGTTCTTA CAGTTATGGT GAAATCCCGT TCGGCATAAC TGACGGACCA
1201 CGATACTGTT ATGTACTTTA CAATGGCACA GCTCTTAAAT ATTTAGGAAC ATTACCACCC
1261 AGTGTAAAGG AAATCGCTAT TAGTAAGTGG GGCCATTTTT ATATTAATGG TTACAATTTC
1321 TTTAGCACAT TTCCTATTGG TTGTATATCT TTTAATTTAA CCACTGGTGT TAGTGGAGCT
1381 TTTTGGACAA TTGCTTACAC ATCGTATACT GAAGCATTAG TACAAGTTGA AAACACAGCT
1441 ATTAAAAATG TGACGTATTG TAACAGTCAC ATTAATAACA TTAAATGTTC TCAACTTACT
1501 GCTAATTTGA ATAATGGATT TTATCCTGTT GCTTCAAGTG AAGTAGGTTT CGTTAATAAG
1561 AGTGTTGTGT TATTACCTAG CTTTTTCACA TACACCGCTG TCAATATAAC CATTGATCTT
1621 GGTATGAAGC TTAGTGGTTA TGGTCAACCC ATAGCCTCGA CACTAAGTAA CATCACACTA
1681 CCAATGCAGG ATAACAATAC TGATGTGTAC TGTATTCGTT CTAACCAATT CTCAGTTTAT
1741 GTTCATTCCA CTTGCAAAAG TTCTTTATGG GACAATATTT TTAATCAAGA CTGCACGGAT
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Figure 5 (cont'd.)

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1801  GTTTTAGAGG CTACAGCTGT TATAAAAACT GGTACTTGTC CTTTCTCATT TGATAAATTG

1861  AACAATTACT TGACTTTTAA CAAGTTCTGT TTGTCGTTGA GTCCTGTTGG TGCTAATTGC

1921  AAGTTTGATG TTGCTGCACG TACAAGAACC AATGAGCAGG TTGTTAGAAG TCTATATGTA

1981  ATATATGAAG AAGGAGACAA CATAGTGGGT GTACCGTCTG ATAATAGCGG TCTGCACGAT

2041  TTGTCTGTGC TACACCTAGA CTCCTGTACA GATTACAATA TATATGGTAG AACTGGTGTT

2101  GGTATTATTA GACGAACTAA CAGTACGCTA CTTAGTGGCT TATATTACAC ATCACTATCA

2161  GGTGATTTGT TAGGCTTTAA AAATGTTAGT GATGGTGTCA TTTATTCTGT GACGCCATGT

2221  GATGTAAGCG CACAAGCGGC TGTTATTGAT GGTGCCATAG TTGGAGCTAT GACTTCCATT

2281  AACAGTGAAC TGTTAGGTCT AACACATTGG ACAACGACAC CTAATTTTTA TTACTACTCT

2341  ATATATAATT ACACAAGTGA GAGGACTCGT GGCACTGCAA TTGACAGTAA  CGATGTTGAT

2401  TGTGAACCTG TCATAACCTA TTCTAATATA GGTGTTTGTA AAAATGGTGC TTTGGTTTTT

2461  ATTAACGTCA CACATTCTGA CGGAGACGTG CAACCAATTA GCACTGGTAA TGTCACGATA

2521  CCTACAAATT TTACCATATC TGTGCAAGTT GAATACATGC AGGTTTACAC TACACCAGTA

2581  TCAATAGATT GTGCAAGATA CGTTTGTAAT GGTAACCCTA GATGTAACAA ATTGTTAACA

2641  CAATATGTGT CTGCATGTCA AACTATTGAA CAAGCACTTG CAATGGGTGC CAGACTTGAA

2701  AACATGGAGG TTGATTCCAT GTTGTTTGTC TCGGAAAATG CCCTTAAATT GGCATCTGTT

2761  GAGGCGTTCA ATAGTACAGA AAATTTAGAT CCTATTTACA AAGAATGGCC TAGCATAGGT

2821  GGTTCTTGGC TAGGAGGTCT AAAAGATATA CTACCGTCCC ATAATAGCAA ACGTAAGTAT

2881  GGTTCTGCTA TAGAAGATTT GCTTTTTGAT AAAGTTGTAA CATCTGGTTT AGGTACAGTT

2941  GATGAAGATT ATAAACGTTG TACTGGTGGT TACGACATAG CAGACTTGGT GTGTGCTCAA

3001  TATTACAATG GCATCATGGT TCTACCAGGT GTAGCTAATG CTGACAAGAT GACTATGTAC

3061  ACAGCATCAC TTGCAGGTGG TATAACATTA GGTGCACTTG GTGGTGGCGC CGTGGCTATA

3121  CCTTTTGCAG TAGCAGTACA GGCTAGACTT AATTATGTTG CTCTACAAAC TGATGTATTG

3181  AATAAAAACC AACAGATCCT GGCTAATGCT TTCAATCAAG CTATTGGTAA CATTACACAG

3241  GCTTTTGGTA AGGTTAATGA TGCTATACAT CAAACATCAC AAGGTCTTGC CACTGTTGCT

3301  AAAGCGTTGG CAAAAGTGCA AGATGTTGTC AACACACAAG GCAAGCTTT AAGTCACCTT

3361  ACAGTACAAT TGCAAAATAA TTTTCAAGCC ATTAGTAGTT CTATTAGTGA TATTTATAAC

3421  AGGCTTGACG AACTGAGTGC TGATGCACAA GTTGATAGGC TGATTACAGG TAGACTTACA

3481  GCACTTAATG CATTTGTGTC TCAGACTCTA ACCAGACAAG CAGAGGTTAG GGCTAGTAGA

3541  CAACTTGCCA AAGACAAGGT TAATGAATGT GTTAGGTCTC AGTCTCAGAG ATTCGGATTC
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Figure 5 (cont'd.)

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3601 TGTGGTAATG GTACACATTT GTTTTCACTA GCAAATGCAG CACCAAATGG CATGATTTTC
3661 TTTCATACAG TACTATTACC AACAGCTTAT GAAACTGTAA CAGCTTGGTC AGGTATTTGT
3721 GCTTCAGATG GCGATCGCAC TTTCGGACTT GTCGTTAAAG ATGTGCAGTT GACGTTGTTT
3781 CGTAATCTAG ATGACAAGTT CTATTTGACC CCCAGAACTA TGTATCAGCC TAGAGTTGCA
3841 ACTAGTTCTG ATTTTGTTCA AATTGAAGGG TGTGATGTGT TGTTTGTCAA CGCGACTGTA
3901 ATTGATTTGC CTAGTATTAT ACCTGACTAT ATTGACATTA ATCAAACTGT TCAAGACATA
3961 TTAGAAAATT ACAGACCAAA CTGGACTGTA CCTGAATTTA CACTTGATAT TTTCAACGCA
4021 ACCTATTTAA ATCTGACTGG TGAAATTGAT GACTTAGAGT TTAGGTCAGA AAAGCTACAT
4081 AACACTACAG TAGAACTTGC CATTCTCATT GATAACATTA ATAATACATT AGTCAATCTT
4141 GAATGGCTCA ATAGAATTGA AACTTATGTA AAATGGCCTT GGTATGTGTG GCTACTGATA
4201 GGTTTAGTAG TAGTATTTTG CATACCATTA CTGCTATTTT GCTGTTTTAG CACAGGTTGT
4261 TGTGGATGCA TAGGTTGTTT AGGAAGTTGT TGTCACTCTA TATGTAGTAG AAGACAATTT
4321 GAAAATTATG AACCAATTGA AAAAGTGCAT GTCCACTAA
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Figure 6

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   1 GAGCTCGCGG CCGCCTATCA AAAGTCTTAA TGAGTTAGGT GTAGATAGTA TAGATATTAC
  61 TACAAAGGTA TTCATATTTC CTATCAATTC TAAAGTAGAT GATATTAATA ACTCAAAGAT
 121 GATGATAGTA GATAATAGAT ACGCTCATAT AATGACTGCA AATTTGGACG GTTCACATTT
 181 TAATCATCAC GCGTTCATAA GTTTCAACTG CATAGATCAA AATCTCACTA AAAAGATAGC
 241 CGATGTATTT GAGAGAGATT GGACATCTAA CTACGCTAAA GAAATTACAG TTATAAATAA
 301 TACATAATGG ATTTTGTTAT CATCAGTTAT ATTTAACATA AGTACAATAA AAAGTATTAA
 361 ATAAAAATAC TTACTTACGA AAAAATGACT AATTAGCTAT AAAAACCCTT AATTAATTAG
 421 TTATTAGACA AGGTGAAAAC GAAACTATTT GTAGCTTAAT TAATTAGAGC TTCTTTATTC
 481 TATACTTAAA AAGTGAAAAT AAATACAAAG GTTCTTGAGG GTTGTGTTAA ATTGAAAGCG
 541 AGAAATAATC ATAAATTATT TCATTATCGA TCCGTTAAGT TTGTATCGTA ATGATTGTGC
 601 TCGTAACTTG CCTCTTGTTG TTATGTTCAT ACCACACAGT TTTGAGTACA ACAAATAATG
 661 AATGCATACA AGTTAACGTA ACACAATTGG CTGGCAATGA AAACCTTATC AGAGATTTTC
 721 TGTTTAGTAA CTTTAAAGAA GAAGGAAGTG TAGTTGTTGG TGGTTATTAC CCTACAGAGG
 781 TGTGGTACAA CTGCTCTAGA ACAGCTCGAA CTACTGCCTT TCAGTATTTT AATAATATAC
 841 ATGCCTTTTA TTTTGTTATG GAAGCCATGG AAAATAGCAC TGGTAATGCA CGTGGTAAAC
 901 CATTATTATT TCATGTGCAT GGTGAGCCTG TTAGTGTTAT TATATCGGCT TATAGGGATG
 961 ATGTGCAACA AAGGCCCCTT TTAAACATG GGTTAGTGTG CATAACTAAA AATCGCCATA
1021 TTAACTATGA ACAATTCACC TCCAACCAGT GGAATTCCAC ATGTACGGGT GCTGACAGAA
1081 AAATTCCTTT CTCTGTCATA CCCACGGACA ATGGAACAAA AATCTATGGT CTTGAGTGGA
1141 ATGATGACTT TGTTACAGCT TATATTAGTG GTCGTTCTTA TCACTTGAAC ATCAATACTA
1201 ATTGGTTTAA CAATGTCACA CTTTTGTATT CACGCTCAAG CACTGCTACC TGGGAATACA
1261 GTGCTGCATA TGCTTACCAA GGTGTTTCTA ACTTCACTTA TTACAAGTTA AATAACACCA
1321 ATGGTCTAAA AACCTATGAA TTATGTGAAG ATTATGAACA TTGCACTGGC TATGCTACCA
1381 ATGTATTTGC TCCGACATCA GGTGGTTACA TACCTGATGG ATTTAGTTTT AACAATTGGT
1441 TCTTGCTTAC AAATAGTTCC ACTTTTGTTA GTGGCAGGTT TGTAACAAAT CAACCATTAT
1501 TGATTAATTG CTTGTGGCCA GTGCCCAGTT TTGGTGTAGC AGCACAAGAA TTTTGTTTTG
1561 AAGGTGCACA GTTTAGCCAA TGTAATGGTG TGTCTTTAAA TAACACAGTG GATGTTATTA
1621 GATTCAACCT TAATTTCACT GCAGATGTAC AATCTGGTAT GGGTGCTACA GTATTTTCAC
1681 TGAATACAAC AGGTGGTGTC ATTCTTGAAA TTTCATGTTA TAGTGACACA GTGAGTGAGT
1741 CTAGTTCTTA CAGTTATGGT GAAATCCCGT TCGGCATAAC TGACGGACCA CGATACTGTT
```

Figure 6 (cont'd.)

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1801 ATGTACTTTA CAATGGCACA GCTCTTAAAT ATTTAGGAAC ATTACCACCC  AGTGTAAAGG

1861 AAATCGCTAT TAGTAAGTGG GGCCATTTCT ATATTAATGG TTACAATTTC TTTAGCACAT

1921 TTCCTATTGG TTGTATATCT TTTAATTTAA CCACTGGTGT TAGTGGAGCT TTTTGGACAA

1981 TTGCTTACAC ATCGTATACT GAAGCATTAG TACAAGTTGA AAACACAGCT ATTAAAAATG

2041 TGACGTATTG TAACAGTCAC ATTAATAACA TTAAATGTTC TCAACTTACT GCTAATTTGA

2101 ATAATGGATT TTATCCTGTT GCTTCAAGTG AAGTAGGTTT CGTTAATAAG AGTGTTGTGT

2161 TATTACCTAG CTTTTTCACA TACACCGCTG TCAATATAAC CATTGATCTT GGTATGAAGC

2221 TTAGTGGTTA TGGTCAACCC ATAGCCTCGA CACTAAGTAA CATCACACTA CCAATGCAGG

2281 ATAACAATAC TGATGTGTAC TGTATTCGTT CTAACCAATT CTCAGTTTAT GTTCATTCCA

2341 CTTGCAAAAG TTCTTTATGG GACAATATTT TTAATCAAGA CTGCACGGAT GTTTTAGAGG

2401 CTACAGCTGT TATAAAAACT GGTACTTGTC CTTTCTCATT TGATAAATTG AACAATTACT

2461 TGACTTTTAA CAAGTTCTGT TTGTCGTTGA GTCCTGTTGG TGCTAATTGC AAGTTTGATG

2521 TTGCTGCACG TACAAGAACC AATGAGCAGG TTGTTAGAAG TCTATATGTA ATATATGAAG

2581 AAGGAGACAA CATAGTGGGT GTACCGTCTG ATAATAGCGG TCTGCACGAT TTGTCTGTGC

2641 TACACCTAGA CTCCTGTACA GATTACAATA TATATGGTAG AACTGGTGTT GGTATTATTA

2701 GACGAACTAA CAGTACGCTA CTTAGTGGCT TATATTACAC ATCACTATCA GGTGATTTGT

2761 TAGGCTTTAA AAATGTTAGT GATGGTGTCA TTTATTCTGT GACGCCATGT GATGTAAGCG

2821 CACAAGCGGC TGTTATCGAT GGTGCCATAG TTGGAGCTAT GACTTCCATT AACAGTGAAC

2881 TGTTAGGCCT AACACATTGG ACAACGACAC CTAATTTCTA TTACTACTCT ATATATAATT

2941 ACACAAGTGA GAGGACTCGT GGCACTGCAA TTGACAGTAA CGATGTTGAT TGTGAACCTG

3001 TCATAACCTA TTCTAATATA GGTGTTTGTA AAAATGGTGC TTTGGTATTT ATTAACGTCA

3061 CACATTCTGA CGGAGACGTG CAACCAATTA GCACTGGTAA TGTCACGATA CCTACAAATT

3121 TTACCATATC TGTGCAAGTT GAATACATGC AGGTTTACAC TACACCAGTA TCAATAGATT

3181 GTGCAAGATA CGTTTGTAAT GGTAACCCTA GATGTAACAA ATTGTTAACA CAATATGTGT

3241 CTGCATGTCA AACTATTGAA CAAGCACTTG CAATGGGTGC CAGACTTGAA AACATGGAGG

3301 TTGATTCCAT GTTGTTTGTC TCGGAAAATG CCCTTAAATT GGCATCTGTT GAGGCGTTCA

3361 ATAGTACAGA AAATTTAGAT CCTATTTACA AGAATGGCC TAGCATAGGT GGTTCTTGGC

3421 TAGGAGGTCT AAAAGATATA CTACCGTCCC ATAATAGCAA ACGTAAGTAT GGTTCTGCTA

3481 TAGAAGATTT GCTTTTTGAT AAAGTTGTAA CATCTGGTTT AGGTACAGTT GATGAAGATT

3541 ATAAACGTTG TACTGGTGGT TACGACATAG CAGACTTGGT GTGTGCTCAA TATTACAATG
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Figure 6 (cont'd.)

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3601 GCATCATGGT TCTACCAGGT GTAGCTAATG CTGACAAGAT GACTATGTAC ACAGCATCAC

3661 TTGCAGGTGG TATAACATTA GGTGCACTTG GTGGTGGCGC CGTGGCTATA CCTTTTGCAG

3721 TAGCAGTACA GGCTAGACTT AATTATGTTG CTCTACAAAC TGATGTATTG AATAAAAACC

3781 AACAGATCCT GGCTAATGCT TTCAATCAAG CTATTGGTAA CATTACACAG GCTTTTGGTA

3841 AGGTTAATGA TGCTATACAT CAAACATCAC AAGGTCTTGC CACTGTTGCT AAAGCGTTGG

3901 CAAAAGTGCA AGATGTTGTC AACACACAAG GGCAAGCTTT AAGTCACCTT ACAGTACAAT

3961 TGCAAATAA TTTTCAAGCC ATTAGTAGTT CTATTAGTGA TATTTATAAC AGGCTTGACG

4021 AACTGAGTGC TGATGCACAA GTTGATAGGC TGATTACAGG TAGACTTACA GCACTTAATG

4081 CATTTGTGTC TCAGACTCTA ACCAGACAAG CAGAGGTTAG GGCTAGTAGA CAACTTGCCA

4141 AAGACAAGGT TAATGAATGT GTTAGGTCTC AGTCTCAGAG ATTCGGATTC TGTGGTAATG

4201 GTACACATTT GTTTTCACTA GCAAATGCAG CACCAAATGG CATGATTTTC TTTCATACAG

4261 TACTATTACC AACAGCTTAT GAAACTGTAA CAGCTTGGTC AGGTATTTGT GCTTCAGATG

4321 GCGATCGCAC TTTCGGACTT GTCGTTAAAG ATGTGCAGTT GACGTTGTTT CGTAATCTAG

4381 ATGACAAGTT CTATTTGACC CCCAGAACTA TGTATCAGCC TAGAGTTGCA ACTAGTTCTG

4441 ATTTTGTTCA AATTGAAGGG TGTGATGTGT TGTTTGTCAA CGCGACTGTA ATTGATTTGC

4501 CTAGTATTAT ACCTGACTAT ATTGACATTA ATCAAACTGT TCAAGACATA TTAGAAAATT

4561 ACAGACCAAA CTGGACTGTA CCTGAATTTA CACTTGATAT TTTCAACGCA ACCTATTTAA

4621 ATCTGACTGG TGAAATTGAT GACTTAGAGT TTAGGTCAGA AAAGCTACAT AACACTACAG

4681 TAGAACTTGC CATTCTCATT GATAACATTA ATAATACATT AGTCAATCTT GAATGGCTCA

4741 ATAGAATTGA AACTTATGTA AAATGGCCTT GGTATGTGTG CTACTGATA GGTTTAGTAG

4801 TAGTATTTTG CATACCATTA CTGCTATTTT GCTGTTTTAG CACAGGTTGT TGTGGATGCA

4861 TAGGTTGTTT AGGAAGTTGT TGTCACTCTA TATGTAGTAG AAGACAATTT GAAAATTATG

4921 AACCAATTGA AAAAGTGCAT GTCCACAAGG TACAATTCTT TTTATTGATT AACTAGTCAA

4981 ATGAGTATAT ATAATTGAAA AAGTAAAATA TAAATCATAT AATAATGAAA CGAAATATCA

5041 GTAATAGACA GGAACTGGCA GATTCTTCTT CTAATGAAGT AAGTACTGCT AAATCTCCAA

5101 AATTAGATAA AAATGATACA GCAAATACAG CTTCATTCAA CGAATTACCT TTTAATTTTT

5161 TCAGACACAC CTTATTACAA ACTAACTAAG TCAGATGATG AGAAAGTAAA TATAAATTTA

5221 ACTTATGGGT ATAATATAAT AAAGATTCAT GATATTAATA ATTTACTTAA CGATGTTAAT

5281 AGACTTATTC CATCAACCCC TTCAAACCTT TCTGGATATT ATAAAATACC AGTTAATGAT

5341 ATTAAAATAG ATTGTTTAAG AGATGTAAAT AATTATTTGG AGGTAAAGGA TATAAAATTA
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Figure 6 (cont'd.)

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5401 GTCTATCTTT CACATGGAAA TGAATTACCT AATATTAATA ATTATGATAG GAATTTTTTA
5461 GGATTTACAG CTGTTATATG TATCAACAAT ACAGGCAGAT CTATGGTTAT GGTAAAACAC
5521 TGTAACGGGA AGCAGCATTC TATGGTAACT GGCCTATGTT TAATAGCCAG ATCATTTTAC
5581 TCTATAAACA TTTTACCACA AATAATAGGA TCCTCTAGAT ATTTAATATT ATATCTAACA
5641 ACAACAAAAA AATTTAACGA TGTATGGCCA GAAGTATTTT CTACTAATAA AGATAAAGAT
5701 AGTCTATCTT ATCTACAAGA TATGAAAGAA GATAATCATT TAGTAGTAGC TACTAATATG
5761 GAAAGAAATG TATACAAAAA CGTGGAAGCT TTTATATTAA ATAGCATATT ACTAGAAGAT
5821 TTAAAATCTA GACTTAGTAT AACAAAACAG TTAAATGCCA ATATCGATTC TATATTTCAT
5881 CATAACAGTA GTACATTAAT CAGTGATATA CTGAAACGAT CTACAGACTC AACTATGCAA
5941 GGAATAAGCA ATATGCCAAT TATGTCTAAT ATTTTAACTT TAGAACTAAA ACGTTCTACC
6001 AATACTAAAA ATAGGATACG TGATAGGCTG TTAAAAGCTG CAATAAATAG TAAGGATGTA
6061 GAAGAAATAC TTTGTTCTAT ACCTTCGGAG GAAAGAACTT TAGAACAACT TAAGTTTAAT
6121 CAAACTTGTA TTTATGAAGG TACC
```

Figure 7

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   1 GAGCTCGCGG CCGCCTATCA AAAGTCTTAA TGAGTTAGGT GTAGATAGTA TAGATATTAC
  61 TACAAAGGTA TTCATATTTC CTATCAATTC TAAAGTAGAT GATATTAATA ACTCAAAGAT
 121 GATGATAGTA GATAATAGAT ACGCTCATAT AATGACTGCA AATTTGGACG GTTCACATTT
 181 TAATCATCAC GCGTTCATAA GTTTCAACTG CATAGATCAA AATCTCACTA AAAAGATAGC
 241 CGATGTATTT GAGAGAGATT GGACATCTAA CTACGCTAAA GAAATTACAG TTATAAATAA
 301 TACATAATGG ATTTTGTTAT CATCAGTTAT ATTTAACATA AGTACAATAA AAAGTATTAA
 361 ATAAAAATAC TTACTTACGA AAAAATGACT AATTAGCTAT AAAAACCCTT AATTAATTAG
 421 TTATTAGACA AGGTGAAAAC GAAACTATTT GTAGCTTAAT TAATTAGAGC TTCTTTATTC
 481 TATACTTAAA AAGTGAAAAT AAATACAAAG GTTCTTGAGG GTTGTGTTAA ATTGAAAGCG
 541 AGAAATAATC ATAAATTATT TCATTATCGA TCCGTTAAGT TTGTATCGTA ATGACAACAA
 601 ATAATGAATG CATACAAGTT AACGTAACAC AATTGGCTGG CAATGAAAAC CTTATCAGAG
 661 ATTTTCTGTT TAGTAACTTT AAAGAAGAAG GAAGTGTAGT TGTTGGTGGT TATTACCCTA
 721 CAGAGGTGTG GTACAACTGC TCTAGAACAG CTCGAACTAC TGCCTTTCAG TATTTTAATA
 781 ATATACATGC CTTTTATTTT GTTATGGAAG CCATGGAAAA TAGCACTGGT AATGCACGTG
 841 GTAAACCATT ATTATTTCAT GTGCATGGTG AGCCTGTTAG TGTTATTATA TCGGCTTATA
 901 GGGATGATGT GCAACAAGG CCCCTTTTAA AACATGGGTT AGTGTGCATA ACTAAAAATC
 961 GCCATATTAA CTATGAACAA TTCACCTCCA ACCAGTGGAA TTCCACATGT ACGGGTGCTG
1021 ACAGAAAAAT TCCTTTCTCT GTCATACCCA CGGACAATGG AACAAAAATC TATGGTCTTG
1081 AGTGGAATGA TGACTTTGTT ACAGCTTATA TTAGTGGTCG TTCTTATCAC TTGAACATCA
1141 ATACTAATTG GTTTAACAAT GTCACACTTT TGTATTCACG CTCAAGCACT GCTACCTGGG
1201 AATACAGTGC TGCATATGCT TACCAAGGTG TTTCTAACTT CACTTATTAC AAGTTAAATA
1261 ACACCAATGG TCTAAAAACC TATGAATTAT GTGAAGATTA TGAACATTGC ACTGGCTATG
1321 CTACCAATGT ATTTGCTCCG ACATCAGGTG GTTACATACC TGATGGATTT AGTTTTAACA
1381 ATTGGTTCTT GCTTACAAAT AGTTCCACTT TTGTTAGTGG CAGGTTTGTA ACAAATCAAC
1441 CATTATTGAT TAATTGCTTG TGGCCAGTGC CCAGTTTTGG TGTAGCAGCA CAAGAATTTT
1501 GTTTTGAAGG TGCACAGTTT AGCCAATGTA ATGGTGTGTC TTTAAATAAC ACAGTGGATG
1561 TTATTAGATT CAACCTTAAT TTCACTGCAG ATGTACAATC TGGTATGGGT GCTACAGTAT
1621 TTTCACTGAA TACAACAGGT GGTGTCATTC TTGAAATTTC ATGTTATAGT GACACAGTGA
1681 GTGAGTCTAG TTCTTACAGT TATGGTGAAA TCCCGTTCGG CATAACTGAC GGACCACGAT
1741 ACTGTTATGT ACTTTACAAT GGCACAGCTC TTAAATATTT AGGAACATTA CCACCCAGTG
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Figure 7 (cont'd.)

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1801 TAAAGGAAAT CGCTATTAGT AAGTGGGGCC ATTTCTATAT TAATGGTTAC  AATTTCTTTA
1861 GCACATTTCC TATTGGTTGT ATATCTTTTA ATTTAACCAC TGGTGTTAGT GGAGCTTTTT
1921 GGACAATTGC TTACACATCG TATACTGAAG CATTAGTACA AGTTGAAAAC ACAGCTATTA
1981 AAAATGTGAC GTATTGTAAC AGTCACATTA ATAACATTAA ATGTTCTCAA CTTACTGCTA
2041 ATTTGAATAA TGGATTTTAT CCTGTTGCTT CAAGTGAAGT AGGTTTCGTT AATAAGAGTG
2101 TTGTGTTATT ACCTAGCTTT TTCACATACA CCGCTGTCAA TATAACCATT GATCTTGGTA
2161 TGAAGCTTAG TGGTTATGGT CAACCCATAG CCTCGACACT AAGTAACATC ACACTACCAA
2221 TGCAGGATAA CAATACTGAT GTGTACTGTA TTCGTTCTAA CCAATTCTCA GTTTATGTTC
2281 ATTCCACTTG CAAAAGTTCT TTATGGGACA ATATTTTTAA TCAAGACTGC ACGGATGTTT
2341 TAGAGGCTAC AGCTGTTATA AAAACTGGTA CTTGTCCTTT CTCATTTGAT AAAATTGAACA
2401 ATTACTTGAC TTTTAACAAG TTCTGTTTGT CGTTGAGTCC TGTTGGTGCT AATTGCAAGT
2461 TTGATGTTGC TGCACGTACA AGAACCAATG AGCAGGTTGT TAGAAGTCTA TATGTAATAT
2521 ATGAAGAAGG AGACAACATA GTGGGTGTAC CGTCTGATAA TAGCGGTCTG CACGATTTGT
2581 CTGTGCTACA CCTAGACTCC TGTACAGATT ACAATATATA TGGTAGAACT GGTGTTGGTA
2641 TTATTAGACG AACTAACAGT ACGCTACTTA GTGGCTTATA TTACACATCA CTATCAGGTG
2701 ATTTGTTAGG CTTTAAAAAT GTTAGTGATG GTGTCATTTA TTCTGTGACG CCATGTGATG
2761 TAAGCGCACA AGCGGCTGTT ATCGATGGTG CCATAGTTGG AGCTATGACT TCCATTAACA
2821 GTGAACTGTT AGGCCTAACA CATTGGACAA CGACACCTAA TTTCTATTAC TACTCTATAT
2881 ATAATTACAC AAGTGAGAGG ACTCGTGGCA CTGCAATTGA CAGTAACGAT GTTGATTGTG
2941 AACCTGTCAT AACCTATTCT AATATAGGTG TTTGTAAAAA TGGTGCTTTG GTATTTATTA
3001 ACGTCACACA TTCTGACGGA GACGTGCAAC CAATTAGCAC TGGTAATGTC ACGATACCTA
3061 CAAATTTTAC CATATCTGTG CAAGTTGAAT ACATGCAGGT TTACACTACA CCAGTATCAA
3121 TAGATTGTGC AAGATACGTT TGTAATGGTA ACCCTAGATG TAACAAATTG TTAACACAAT
3181 ATGTGTCTGC ATGTCAAACT ATTGAACAAG CACTTGCAAT GGGTGCCAGA CTTGAAAACA
3241 TGGAGGTTGA TTCCATGTTG TTTGTCTCGG AAAATGCCCT TAAATTGGCA TCTGTTGAGG
3301 CGTTCAATAG TACAGAAAAT TTAGATCCTA TTTACAAAGA ATGGCCTAGC ATAGGTGGTT
3361 CTTGGCTAGG AGGTCTAAAA GATATACTAC CGTCCCATAA TAGCAAACGT AAGTATGGTT
3421 CTGCTATAGA AGATTTGCTT TTTGATAAAG TTGTAACATC TGGTTTAGGT ACAGTTGATG
3481 AAGATTATAA ACGTTGTACT GGTGGTTACG ACATAGCAGA CTTGGTGTGT GCTCAATATT
3541 ACAATGGCAT CATGGTTCTA CCAGGTGTAG CTAATGCTGA CAAGATGACT ATGTACACAG
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Figure 7 (cont'd.)

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3601 CATCACTTGC AGGTGGTATA ACATTAGGTG CACTTGGTGG TGGCGCCGTG GCTATACCTT

3661 TTGCAGTAGC AGTACAGGCT AGACTTAATT ATGTTGCTCT ACAAACTGAT GTATTGAATA

3721 AAAACCAACA GATCCTGGCT AATGCTTTCA ATCAAGCTAT TGGTAACATT ACACAGGCTT

3781 TTGGTAAGGT TAATGATGCT ATACATCAAA CATCACAAGG TCTTGCCACT GTTGCTAAAG

3841 CGTTGGCAAA AGTGCAAGAT GTTGTCAACA CACAAGGGCA AGCTTTAAGT CACCTTACAG

3901 TACAATTGCA AAATAATTTT CAAGCCATTA GTAGTTCTAT TAGTGATATT TATAACAGGC

3961 TTGACGAACT GAGTGCTGAT GCACAAGTTG ATAGGCTGAT TACAGGTAGA CTTACAGCAC

4021 TTAATGCATT TGTGTCTCAG ACTCTAACCA GACAAGCAGA GGTTAGGGCT AGTAGACAAC

4081 TTGCCAAAGA CAAGGTTAAT GAATGTGTTA GGTCTCAGTC TCAGAGATTC GGATTCTGTG

4141 GTAATGGTAC ACATTTGTTT TCACTAGCAA ATGCAGCACC AAATGGCATG ATTTTCTTTC

4201 ATACAGTACT ATTACCAACA GCTTATGAAA CTGTAACAGC TTGGTCAGGT ATTTGTGCTT

4261 CAGATGGCGA TCGCACTTTC GGACTTGTCG TTAAAGATGT GCAGTTGACG TTGTTTCGTA

4321 ATCTAGATGA CAAGTTCTAT TTGACCCCCA GAACTATGTA TCAGCCTAGA GTTGCAACTA

4381 GTTCTGATTT TGTTCAAATT GAAGGGTGTG ATGTGTTGTT TGTCAACGCG ACTGTAATTG

4441 ATTTGCCTAG TATTATACCT GACTATATTG ACATTAATCA AACTGTTCAA GACATATTAG

4501 AAAATTACAG ACCAAACTGG ACTGTACCTG AATTTACACT TGATATTTTC AACGCAACCT

4561 ATTTAAATCT GACTGGTGAA ATTGATGACT TAGAGTTTAG GTCAGAAAAG CTACATAACA

4621 CTACAGTAGA ACTTGCCATT CTCATTGATA ACATTAATAA TACATTAGTC AATCTTGAAT

4681 GGCTCAATAG AATTGAAACT TATGTAAAAT GGCCTTGGTA TGTGTGGCTA CTGATAGGTT

4741 TAGTAGTAGT ATTTTGCATA CCATTACTGC TATTTGCTG TTTTAGCACA GGTTGTTGTG

4801 GATGCATAGG TTGTTTAGGA AGTTGTTGTC ACTCTATATG TAGTAGAAGA CAATTTGAAA

4861 ATTATGAACC AATTGAAAAA GTGCATGTCC ACAAGGTACA ATTCTTTTTA TTGATTAACT

4921 AGTCAAATGA GTATATATAA TTGAAAAAGT AAAATATAAA TCATATAATA ATGAAACGAA

4981 ATATCAGTAA TAGACAGGAA CTGGCAGATT CTTCTTCTAA TGAAGTAAGT ACTGCTAAAT

5041 CTCCAAAATT AGATAAAAAT GATACAGCAA ATACAGCTTC ATTCAACGAA TTACCTTTTA

5101 ATTTTTTCAG ACACACCTTA TTACAAACTA ACTAAGTCAG ATGATGAGAA AGTAAATATA

5161 AATTTAACTT ATGGGTATAA TATAATAAAG ATTCATGATA TTAATAATTT ACTTAACGAT

5221 GTTAATAGAC TTATTCCATC AACCCCTTCA AACCTTTCTG GATATTATAA AATACCAGTT

5281 AATGATATTA AAATAGATTG TTTAAGAGAT GTAAATAATT ATTTGGAGGT AAAGGATATA

5341 AAATTAGTCT ATCTTTCACA TGGAAATGAA TTACCTAATA TTAATAATTA TGATAGGAAT
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Figure 7 (cont'd.)

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5401 TTTTTAGGAT TTACAGCTGT TATATGTATC AACAATACAG GCAGATCTAT GGTTATGGTA

5461 AAACACTGTA ACGGGAAGCA GCATTCTATG GTAACTGGCC TATGTTTAAT AGCCAGATCA

5521 TTTTACTCTA TAAACATTTT ACCACAAATA ATAGGATCCT CTAGATATTT AATATTATAT

5581 CTAACAACAA CAAAAAAATT TAACGATGTA TGGCCAGAAG TATTTTCTAC TAATAAAGAT

5641 AAAGATAGTC TATCTTATCT ACAAGATATG AAAGAAGATA ATCATTTAGT AGTAGCTACT

5701 AATATGGAAA GAAATGTATA CAAAAACGTG GAAGCTTTTA TATTAAATAG CATATTACTA

5761 GAAGATTTAA AATCTAGACT TAGTATAACA AAACAGTTAA ATGCCAATAT CGATTCTATA

5821 TTTCATCATA ACAGTAGTAC ATTAATCAGT GATATACTGA AACGATCTAC AGACTCAACT

5881 ATGCAAGGAA TAAGCAATAT GCCAATTATG TCTAATATTT TAACTTTAGA ACTAAAACGT

5941 TCTACCAATA CTAAAAATAG GATACGTGAT AGGCTGTTAA AAGCTGCAAT AAATAGTAAG

6001 GATGTAGAAG AAATACTTTG TTCTATACCT TCGGAGGAAA GAACTTTAGA ACAACTTAAG

6061 TTTAATCAAA CTTGTATTTA TGAAGGTACC
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Figure 8

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   1 GAGCTCGCGG CCGCCTATCA AAAGTCTTAA TGAGTTAGGT GTAGATAGTA TAGATATTAC
  61 TACAAAGGTA TTCATATTTC CTATCAATTC TAAAGTAGAT GATATTAATA ACTCAAAGAT
 121 GATGATAGTA GATAATAGAT ACGCTCATAT AATGACTGCA AATTTGGACG GTTCACATTT
 181 TAATCATCAC GCGTTCATAA GTTTCAACTG CATAGATCAA AATCTCACTA AAAAGATAGC
 241 CGATGTATTT GAGAGAGATT GGACATCTAA CTACGCTAAA GAAATTACAG TTATAAATAA
 301 TACATAATGG ATTTGTTAT CATCAGTTAT ATTTAACATA AGTACAATAA AAAGTATTAA
 361 ATAAAAATAC TTACTTACGA AAAAATGACT AATTAGCTAT AAAAACCCTT AATTAATTAG
 421 TTATTAGACA AGGTGAAAAC GAAACTATTT GTAGCTTAAT TAATTAGAGC TTCTTTATTC
 481 TATACTTAAA AAGTGAAAAT AAATACAAAG GTTCTTGAGG GTTGTGTTAA ATTGAAAGCG
 541 AGAAATAATC ATAAATTATT TCATTATCGA TCCGTTAAGT TTGTATCGTA ATGGGTAACC
 601 CTAGATGTAA CAAATTGTTA ACACAATATG TGTCTGCATG TCAAACTATT GAACAAGCAC
 661 TTGCAATGGG TGCCAGACTT GAAAACATGG AGGTTGATTC CATGTTGTTT GTCTCGGAAA
 721 ATGCCCTTAA ATTGGCATCT GTTGAGGCGT TCAATAGTAC AGAAAATTTA GATCCTATTT
 781 ACAAAGAATG GCCTAGCATA GGTGGTTCTT GGCTAGGAGG TCTAAAAGAT ATACTACCGT
 841 CCCATAATAG CAAACGTAAG TATGGTTCTG CTATAGAAGA TTTGCTTTTT GATAAAGTTG
 901 TAACATCTGG TTTAGGTACA GTTGATGAAG ATTATAAACG TTGTACTGGT GGTTACGACA
 961 TAGCAGACTT GGTGTGTGCT CAATATTACA ATGGCATCAT GGTTCTACCA GGTGTAGCTA
1021 ATGCTGACAA GATGACTATG TACACAGCAT CACTTGCAGG TGGTATAACA TTAGGTGCAC
1081 TTGGTGGTGG CGCCGTGGCT ATACCTTTTG CAGTAGCAGT ACAGGCTAGA CTTAATTATG
1141 TTGCTCTACA AACTGATGTA TTGAATAAAA ACCAACAGAT CCTGGCTAAT GCTTTCAATC
1201 AAGCTATTGG TAACATTACA CAGGCTTTTG GTAAGGTTAA TGATGCTATA CATCAAACAT
1261 CACAAGGTCT TGCCACTGTT GCTAAAGCGT TGGCAAAAGT GCAAGATGTT GTCAACACAC
1321 AAGGGCAAGC TTTAAGTCAC CTTACAGTAC AATTGCAAAA TAATTTTCAA GCCATTAGTA
1381 GTTCTATTAG TGATATTTAT AACAGGCTTG ACGAACTGAG TGCTGATGCA CAAGTTGATA
1441 GGCTGATTAC AGGTAGACTT ACAGCACTTA ATGCATTTGT GTCTCAGACT CTAACCAGAC
1501 AAGCAGAGGT TAGGGCTAGT AGACAACTTG CCAAAGACAA GGTTAATGAA TGTGTTAGGT
1561 CTCAGTCTCA GAGATTCGGA TTCTGTGGTA ATGGTACACA TTTGTTTTCA CTAGCAAATG
1621 CAGCACCAAA TGGCATGATT TTCTTTCATA CAGTACTATT ACCAACAGCT TATGAAACTG
1681 TAACAGCTTG GTCAGGTATT TGTGCTTCAG ATGGCGATCG CACTTTCGGA CTTGTCGTTA
1741 AAGATGTGCA GTTGACGTTG TTTCGTAATC TAGATGACAA GTTCTATTTG ACCCCCAGAA
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Figure 8 (cont'd.)

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1801 CTATGTATCA GCCTAGAGTT GCAACTAGTT CTGATTTTGT TCAAATTGAA GGGTGTGATG

1861 TGTTGTTTGT CAACGCGACT GTAATTGATT TGCCTAGTAT TATACCTGAC TATATTGACA

1921 TTAATCAAAC TGTTCAAGAC ATATTAGAAA ATTACAGACC AAACTGGACT GTACCTGAAT

1981 TTACACTTGA TATTTTCAAC GCAACCTATT TAAATCTGAC TGGTGAAATT GATGACTTAG

2041 AGTTTAGGTC AGAAAAGCTA CATAACACTA CAGTAGAACT TGCCATTCTC ATTGATAACA

2101 TTAATAATAC ATTAGTCAAT CTTGAATGGC TCAATAGAAT TGAAACTTAT GTAAAATGGC

2161 CTTGGTATGT GTGGCTACTG ATAGGTTTAG TAGTAGTATT TTGCATACCA TTACTGCTAT

2221 TTTGCTGTTT TAGCACAGGT TGTTGTGGAT GCATAGGTTG TTTAGGAAGT TGTTGTCACT

2281 CTATATGTAG TAGAAGACAA TTTGAAAATT ATGAACCAAT TGAAAAAGTG CATGTCCACA

2341 AGGTACAATT CTTTTTATTG ATTAACTAGT CAAATGAGTA TATATAATTG AAAAAGTAAA

2401 ATATAAATCA TATAATAATG AAACGAAATA TCAGTAATAG ACAGGAACTG GCAGATTCTT

2461 CTTCTAATGA AGTAAGTACT GCTAAATCTC CAAAATTAGA TAAAAATGAT ACAGCAAATA

2521 CAGCTTCATT CAACGAATTA CCTTTTAATT TTTTCAGACA CACCTTATTA CAAACTAACT

2581 AAGTCAGATG ATGAGAAAGT AAATATAAAT TTAACTTATG GGTATAATAT AATAAAGATT

2641 CATGATATTA ATAATTTACT TAACGATGTT AATAGACTTA TTCCATCAAC CCCTTCAAAC

2701 CTTTCTGGAT ATTATAAAAT ACCAGTTAAT GATATTAAAA TAGATTGTTT AAGAGATGTA

2761 AATAATTATT TGGAGGTAAA GGATATAAAA TTAGTCTATC TTTCACATGG AAATGAATTA

2821 CCTAATATTA ATAATTATGA TAGGAATTTT TTAGGATTTA CAGCTGTTAT ATGTATCAAC

2881 AATACAGGCA GATCTATGGT TATGGTAAAA CACTGTAACG GGAAGCAGCA TTCTATGGTA

2941 ACTGGCCTAT GTTTAATAGC CAGATCATTT TACTCTATAA ACATTTTACC ACAAATAATA

3001 GGATCCTCTA GATATTTAAT ATTATATCTA ACAACAACAA AAAAATTTAA CGATGTATGG

3061 CCAGAAGTAT TTTCTACTAA TAAAGATAAA GATAGTCTAT CTTATCTACA AGATATGAAA

3121 GAAGATAATC ATTTAGTAGT AGCTACTAAT ATGGAAAGAA ATGTATACAA AAACGTGGAA

3181 GCTTTTATAT TAAATAGCAT ATTACTAGAA GATTTAAAAT CTAGACTTAG TATAACAAAA

3241 CAGTTAAATG CCAATATCGA TTCTATATTT CATCATAACA GTAGTACATT AATCAGTGAT

3301 ATACTGAAAC GATCTACAGA CTCAACTATG CAAGGAATAA GCAATATGCC AATTATGTCT

3361 AATATTTTAA CTTTAGAACT AAAACGTTCT ACCAATACTA AAAATAGGAT ACGTGATAGG

3421 CTGTTAAAAG CTGCAATAAA TAGTAAGGAT GTAGAAGAAA TACTTTGTTC TATACCTTCG

3481 GAGGAAAGAA CTTTAGAACA ACTTAAGTTT AATCAAACTT GTATTTATGA AGGTACC
```

Figure 9

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   1 AGATATTTGT TAGCTTCTGC CGGAGATACC GTGAAAATCT ATTTTCTGGA AGGAAAGGGA
  61 GGTCTTATCT ATTCTGTCAG CAGAGTAGGT TCCTCTAATG ACGAAGACAA TAGTGAATAC
 121 TTGCATGAAG GTCACTGTGT AGAGTTCAAA ACTGATCATC AGTGTTTGAT AACTCTAGCG
 181 TGTACGAGTC CTTCTAACAC TGTGGTTTAT TGGCTGGAAT AAAAGGATAA AGACACCTAT
 241 ACTGATTCAT TTTCATCTGT CAACGTTTCT CTAAGAGATT CATAGGTATT ATTATTACAT
 301 CGATCTAGAA GTCTAATAAC TGCTAAGTAT ATTATTGGAT TTAACGCGCT ATAAACGCAT
 361 CCAAAACCTA CAAATATAGG AGAAGCTTCT CTTATGAAAC TTCTTAAAGC TTTACTCTTA
 421 CTATTACTAC TCAAAGAGA TATTACATTA ATTATGTGAT GAGGCATCCA ACATATAAAG
 481 AAGACTAAAG CTGTAGAAGC TGTTATGAAG AATATCTTAT CAGATATATT AGATGCATTG
 541 TTAGTTCTGT AGATCAGTAA CGTATAGCAT ACGAGTATAA TTATCGTAGG TAGTAGGTAT
 601 CCTAAAATAA ATCTGATACA GATAATAACT TTGTAAATCA ATTCAGCAAT TTCTCTATTA
 661 TCATGATAAT GATTAATACA CAGCGTGTCG TTATTTTTTG TTACGATAGT ATTTCTAAAG
 721 TAAAGAGCAG GAATCCCTAG TATAATAGAA ATAATCCATA TGAAAAATAT AGTAATGTAC
 781 ATATTTCTAA TGTTAACATA TTTATAGGTA AATCCAGGAA GGGTAATTTT TACATATCTA
 841 TATACGCTTA TTACAGTTAT TAAAAATATA CTTGCAAACA TGTTAGAAGT AAAAAAGAAA
 901 GAACTAATTT TACAAAGTGC TTTACCAAAA TGCCAATGGA AATTACTTAG TATGTATATA
 961 ATGTATAAAG GTATGAATAT CACAAACAGC AAATCGGCTA TTCCCAAGTT GAGAAACGGT
1021 ATAATAGATA TATTTCTAGA TACCATTAAT AACCTTATAA GCTTGACGTT TCCTATAATG
1081 CCTACTAAGA AAACTAGAAG ATACATACAT ACTAACGCCA TACGAGAGTA ACTACTCATC
1141 GTATAACTAC TGTTGCTAAC AGTGACACTG ATGTTATAAC TCATCTTTGA TGTGGTATAA
1201 ATGTATAATA ACTATATTAC ACTGGTATTT TATTTCAGTT ATATACTATA TAGTATTAAA
1261 AATTATATTT GTATAATTAT ATTATTATAT TCAGTGTAGA AAGTAAAATA CTATAAATAT
1321 GTATCTCTTA TTTATAACTT ATTAGTAAAG TATGTACTAT TCAGTTATAT TGTTTTATAA
1381 AAGCTAAATG CTACTAGATT GATATAAATG AATATGTAAT AAATTAGTAA TGTAGTATAC
1441 TAATATTAAC TCACATTATG AATACTACTA ATCACGAAGA ATGCAGTAAA ACATATGATA
1501 CAAACATGTT AACAGTTTTA AAAGCCATTA GTAATAAACA GTACAATATA ATTAAGTCTT
1561 TACTTAAAAA AGATATTAAT GTTAATAGAT TATTAACTAG TTATTCTAAC GAAATATATA
1621 AACATTTAGA CATTACATTA TGTAATATAC TTATAGAACG TGCAGCAGAC ATAAACATTA
1681 TAGATAAGAA CAATCGTACA CCGTTGTTTT ATGCGGTAAA GAATAATGAT TATGATATGG
1741 TTAAACTCCT ATTAAAAAAT GGCGCGAATG TAAATTTACA AGATAGTATA GGATATTCAT
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Figure 9 (cont'd.)

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1801 GTCTTCACAT CGCAGGTATA CATAATAGTA ACATAGAAAT AGTAGATGCA TTGATATCAT

1861 ACAAACCAGA TTTAAACTCC CGCGATTGGG TAGGTAGAAC ACCGCTACAT ATCTTCGTGA

1921 TAGAATCTAA CTTTGAAGCT GTGAAATTAT TATTAAAGTC AGGTGCATAT GTAGGTTTGA

1981 AAGACAAATG TAAGCATTTT CCTATACACC ATTCTGTAAT GAAATTAGAT CACTTAATAT

2041 CAGGATTGTT ATTAAAATAT GGAGCAAATC CAAATACAAT TAACGGCAAT GGAAAAACAT

2101 TATTAAGCAT TGCTGTAACA TCTAATAATA CACTACTGGT AGAACAGCTG CTGTTATATG

2161 GAGCAGAAGT TAATAATGGT GGTTATGATG TTCCAGCTCC TATTATATCC GCTGTCAGTG

2221 TTAACAATTA TGATATTGTT AAGATACTGA TACATAATGG TGCGAATATA AATGTATCCA

2281 CGGAAGATGG TAGAACGTCT TTACATACAG CTATGTTTTG GAATAACGCT AAAATAATAG

2341 ATGAGTTGCT TAACTATGGA AGTGACATAA ACAGCGTAGA TACTTATGGT AGAACTCCGT

2401 TATCTTGTTA TCGTAGCTTA AGTTATGATA TCGCTACTAA ACTAATATCA CGTATCATTA

2461 TAACAGATGT CTATCGTGAA GCACCAGTAA ATATCAGCGG ATTTATAATT AATTTAAAAA

2521 CTATAGAAAA TAATGATATA TTCAAATTAA TTAAAGATGA TTGTATTAAA GAGATAAACA

2581 TACTTAAAAG TATAACCCTT AATAAATTTC ATTCATCTGA CATATTTATA CGATATAATA

2641 CTGATATATG TTTATTAACG AGATTTATTC AACATCCAAA GATAATAGAA CTAGACAAAA

2701 AACTCTACGC TTATAAATCT ATAGTCAACG AGAGAAAAAT CAAAGCTACT TACAGGTATT

2761 ATCAAATAAA AAAAGTATTA ACTGTACTAC CTTTTTCAGG ATATTTCTCT ATATTGCCGT

2821 TTGATGTGTT AGTATATATA CTTGAATTCA TCTATGATAA TAATATGTTG GTACTTATGA

2881 GAGCGTTATC ATTAAAATGA AATAAAAAGC ATACAAGCTA TTGCTTCGCT ATCGTTACAA

2941 AATGGCAGGA ATTTTGTGTA AACTAAGCCA CATACTTGCC AATGAAAAAA ATAGTAGAAA

3001 GGATACTATT TTAATGGGAT TAGATGTTAA GGTTCCTTGG GATTATAGTA ACTGGGCATC

3061 TGTTAACTTT TACGACGTTA GGTTAGATAC TGATGTTACA GATTATAATA ATGTTACAAT

3121 AAAATACATG ACAGGATGTG ATATTTTTCC TCATATAACT CTTGGAATAG CAAATATGGA

3181 TCAATGTGAT AGATTTGAAA ATTTCAAAAA GCAAATAACT GATCAAGATT TACAGACTAT

3241 TTCTATAGTC TGTAAAGAAG AGATGTGTTT CCTCAGAGT AACGCCTCTA AACAGTTGGG

3301 AGCGAAAGGA TGCGCTGTAG TTATGAAACT GGAGGTATCT GATGAACTTA GAGCCCTAAG

3361 AAATGTTCTG CTGAATGCGG TACCCTGTTC GAAGGACGTG TTTGGTGATA TCACAGTAGA

3421 TAATCCGTGG AATCCTCACA TAACAGTAGG ATATGTTAAG GAGGACGATG TCGAAAACAA

3481 GAAACGCCTA ATGGAGTGCA TGTCCAAGTT TAGGGGGCAA GAAATACAAG TTCTAGGATG

3541 GTATTAATAA GTATCTAAGT ATTTGGTATA ATTTATTAAA TAGTATAATT ATAACAAATA
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Figure 9 (cont'd.)

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3601 ATAAATAACA TGATAACGGT TTTTATTAGA ATAAAATAGA GATAATATCA TAATGATATA

3661 TAATACTTCA TTACCAGAAA TGAGTAATGG AAGACTTATA AATGAACTGC ATAAAGCTAT

3721 AAGGTATAGA GATATAAATT TAGTAAGGTA TATACTTAAA AAATGCAAAT ACAATAACGT

3781 AAATATACTA TCAACGTCTT TGTATTTAGC CGTAAGTATT TCTGATATAG AAATGGTAAA

3841 ATTATTACTA GAACACGGTG CCGATATTTT AAAATGTAAA AATCCTCCTC TTCATAAAGC

3901 TGCTAGTTTA GATAATACAG AAATTGCTAA ACTACTAATA GATTCTGGCG CTGACATAGA

3961 ACAGATACAT TCTGGAAATA GTCCGTTATA TATTTCTGTA TATAGAAACA ATAAGTCATT

4021 AACTAGATAT TTATTAAAAA AAGGTGTTAA TTGTAATAGA TTCTTTCTAA ATTATTACGA

4081 TGTACTGTAT GATAAGATAT CTGATGATAT GTATAAAATA TTTATAGATT TTAATATTGA

4141 TCTTAATATA CAAACTAGAA ATTTTGAAAC TCCGTTACAT TACGCTATAA AGTATAAGAA

4201 TATAGATTTA ATTAGGATAT TGTTAGATAA TAGTATTAAA ATAGATAAAA GTTTATTTTT

4261 GCATAAACAG TATCTCATAA AGGCACTTAA AAATAATTGT AGTTACGATA TAATAGCGTT

4321 ACTTATAAAT CACGGAGTGC CTATAAACGA ACAAGATGAT TTAGGTAAAA CCCCATTACA

4381 TCATTCGGTA ATTAATAGAA GAAAGATGT AACAGCACTT CTGTTAAATC TAGGAGCTGA

4441 TATAAACGTA ATAGATGACT GTATGGGCAG TCCCTTACAT TACGCTGTTT CACGTAACGA

4501 TATCGAAACA ACAAAGACAC TTTTAGAAAG AGGATCTAAT GTTAATGTGG TTAATAATCA

4561 TATAGATACC GTTCTAAATA TAGCTGTTGC ATCTAAAAAC AAAACTATAG TAAACTTATT

4621 ACTGAAGTAC GGTACTGATA CAAAGTTGGT AGGATTAGAT AAACATGTTA TTCACATAGC

4681 TATAGAAATG AAAGATATTA ATATACTGAA TGCGATCTTA TTATATGGTT GCTATGTAAA

4741 CGTCTATAAT CATAAAGGTT TCACTCCTCT ATACATGGCA GTTAGTTCTA TGAAAACAGA

4801 ATTTGTTAAA CTCTTACTTG ACCACGGTGC TTACGTAAAT GCTAAAGCTA AGTTATCTGG

4861 AAATACTCCT TTACATAAAG CTATGTTATC TAATAGTTTT AATAATATAA AATTACTTTT

4921 ATCTTATAAC GCCGACTATA ATTCTCTAAA TAATCACGGT AATACGCCTC TAACTTGTGT

4981 TAGCTTTTTA GATGACAAGA TAGCTATTAT GATAATATCT AAAATGATGT TAGAAATATC

5041 TAAAAATCCT GAAATAGCTA ATTCAGAAGG TTTTATAGTA AACATGGAAC ATATAAACAG

5101 TAATAAAAGA CTACTATCTA TAAAGAATC ATGCGAAAAA GAACTAGATG TTATAACACA

5161 TATAAAGTTA AATTCTATAT ATTCTTTTAA TATCTTTCTT GACAATAACA TAGATCTTAT

5221 GGTAAAGTTC GTAACTAATC CTAGAGTTAA TAAGATACCT GCATGTATAC GTATATATAG

5281 GGAATTAATA CGGAAAAATA AATCATTAGC TTTTCATAGA CATCAGCTAA TAGTTAAAGC

5341 TGTAAAAGAG AGTAAGAATC TAGGAATAAT AGGTAGGTTA CCTATAGATA TCAAACATAT
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Figure 9 (cont'd.)

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5401 AATAATGGAA CTATTAAGTA ATAATGATTT ACATTCTGTT ATCACCAGCT GTTGTAACCC

5461 AGTAGTATAA AGTGATTTTA TTCAATTACG AAGATAAACA TTAAATTTGT TAACAGATAT

5521 GAGTTATGAG TATTTAACTA AAGTTACTTT AGGTACAAAT AAAATATTAT GTAATATAAT

5581 AGAAAATTAT CTTGAGTCTT CATTTCCATC ACCGTCTAAA TTTATTATTA AAACCTTATT

5641 ATATAAGGCT GTTGAGTTTA GAAATGTAAA TGCTGTAAAA AAAATATTAC AGAATGATAT

5701 TGAATATGTT AAAGTAGATA GTCATGGTGT CTCGCCTTTA CATATTATAG CTATGCCTTC

5761 AAATTTTTCT CTCATAGACG CTGACATGTA TTCAGAATTT AATGAAATTA GTAATAGACT

5821 TCAAAAATCT AAAGATAGTA ACGAATTTCA ACGAGTTAGT CTACTAAGGA CAATTATAGA

5881 ATATGGTAAT GATAGTGATA TTAATAAGTG TCTAACATTA GTAAAAACGG ATATACAGAG

5941 TAACGAAGAG ATAGATATTA TAGATCTTTT GATAAATAAA GGAATAGATA TAAATATTAA

6001 AGACGATTTA GGAAACACAG CTTTGCATTA CTCGTGTGAT TATGCTAAGG GATCAAAGAT

6061 AGCTAAAAAG TTACTAGATT GTGGAGCAGA TCCTAACATA GTTAATGATT TAGGTGTTAC

6121 ACCACTAGCG TGTGCCGTTA ATACTTGCAA CGAGATACTA GTAGATATTC TGTTAAATAA

6181 TGATGCGAAT CCTGATTCAT CTTCCTCATA TTTTTTAGGT ACTAATGTGT TACATACAGC

6241 CGTAGGTACC GGTAATATAG ATATTGTAAG ATCTTTACTT ACGGCTGGTG CCAATCCTAA

6301 TGTAGGAGAT AAATCTGGAG TTACTCCTTT GCACGTTGCT GCAGCTGATA AGACAGTTA

6361 TCTGTTAATG GAGATGCTAC TAGATAGCGG GGCAGATCCA AATATAAAAT GCGCAAACGG

6421 TTTTACTCCT TTGTTTAATG CAGTATATGA TCATAACCGT ATAAAGTTAT TATTTCTTTA

6481 CGGGGCTGAT ATCAATATTA CTGACTCTTA CGGAAATACT CCTCTTACTT ATATGACTAA

6541 TTTTGATAAT AAATATGTAA ATTCAATAAT TATCTTACAA ATATATCTAC TTAAAAAAGA

6601 ATATAACGAT GAAAGATTGT TTCCACCTGG TATGATAAAA AATTTAAACT TTATAGAATC

6661 AAACGATAGT CTTAAAGTTA TAGCTAAAAA GTGTAATTCG TTAATACGCT ATAAGAAAAA

6721 TAAAGACATA GATGCAGATA ACGTATTATT GGAGCTTTTA GAGGAAGAGG AAGAAGATGA

6781 AATAGACAGA TGGCATACTA CATGTAAAAT ATCTTAAATA GTAATTAAAT CATTGAAATA

6841 TTAACTTACA AGATGATCGA GGTCACTTAT TATACTCTTT AATAATGGGT ACAAAGAGTA

6901 TTCATACGTT AGTTAAATCT AACGATGTAA TACGTGTTCG TGAATTAATA AAGGATGATA

6961 GATGTTTGAT AAATAAAAGA AATAGAAGAA TCAGTCACC TGTATATATA GCTATATACA

7021 AAGGACTTTA TGAAATGACT GAAATGTTAT TGCTAAATAA TGCAAGTCTA GATACTAAAA

7081 TACCTTCTTT AATTATAGCA GCTAAAAATA ATGACTTACC TATGATAAAA TTATTGATAC

7141 AATACGGGGC AAAATTAAAT GATATTTATT TAAGGGACAC AGCATTAATG ATAGCTCTCA
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Figure 9 (cont'd.)

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7201 GAAATGGTTA CCTAGATATA GCTGAATATT TACTTTCATT AGGAGCAGAA TTTGTTAAAT
7261 ACAGACATAA GGTAATATAT AAATATCTAT CAAAAGATGC GTATGAATTA CTTTTTAGAT
7321 TTAATTATGA CGTTAATATA ATAGATTGAG A
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Figure 10

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   1 TGAATGTTAA ATGTTATACT TTGGATGAAG CTATAAATAT GCATTGGAAA AATAATCCAT
  61 TTAAAGAAAG GATTCAAATA CTACAAAACC TAAGCGATAA TATGTTAACT AAGCTTATTC
 121 TTAACGACGC TTTAAATATA CACAAATAAA CATAATTTTT GTATAACCTA ACAAATAACT
 181 AAAACATAAA AATAATAAAA GGAAATGTAA TATCGTAATT ATTTTACTCA GGAATGGGGT
 241 TAAATATTTA TATCACGTGT ATATCTATAC TGTTATCGTA TACTCTTTAC AATTACTATT
 301 ACGAATATGC AAGAGATAAT AAGATTACGT ATTTAAGAGA ATCTTGTCAT GATAATTGGG
 361 TACGACATAG TGATAAATGC TATTTCGCAT CGTTACATAA AGTCAGTTGG AAAGATGGAT
 421 TTGACAGATG TAACTTAATA GGTGCAAAAA TGTTAAATAA CAGCATTCTA TCGGAAGATA
 481 GGATACCAGT TATATTATAC AAAAATCACT GGTTGGATAA AACAGATTCT GCAATATTCG
 541 TAAAGATGA AGATTACTGC GAATTTGTAA ACTATGACAA TAAAAAGCCA TTTATCTCAA
 601 CGACATCGTG TAATTCTTCC ATGTTTTATG TATGTGTTTC AGATATTATG AGATTACTAT
 661 AAACTTTTTG TATACTTATA TTCCGTAAAC TATATTAATC ATGAAGAAAA TGAAAAAGTA
 721 TAGAAGCTGT TCACGAGCGG TTGTTGAAAA CAACAAAATT ATACATTCAA GATGGCTTAC
 781 ATATACGTCT GTGAGGCTAT CATGGATAAT GACAATGCAT CTCTAAATAG GTTTTTGGAC
 841 AATGGATTCG ACCCTAACAC GGAATATGGT ACTCTACAAT CTCCTCTTGA AATGGCTGTA
 901 ATGTTCAAGA ATACCGAGGC TATAAAAATC TTGATGAGGT ATGGAGCTAA ACCTGTAGTT
 961 ACTGAATGCA CAACTTCTTG TCTGCATGAT GCGGTGTTGA GAGACGACTA CAAAATAGTG
1021 AAAGATCTGT TGAAGAATAA CTATGTAAAC AATGTTCTTT ACAGCGGAGG CTTTACTCCT
1081 TTGTGTTTGG CAGCTTACCT TAACAAAGTT AATTTGGTTA AACTTCTATT GGCTCATTCG
1141 GCGGATGTAG ATATTTCAAA CACGGATCGG TTAACTCCTC TACATATAGC CGTATCAAAT
1201 AAAAATTTAA CAATGGTTAA ACTTCTATTG AACAAAGGTG CTGATACTGA CTTGCTGGAT
1261 AACATGGGAC GTACTCCTTT AATGATCGCT GTACAATCTG GAAATATTGA AATATGTAGC
1321 ACACTACTTA AAAAAAATAA AATGTCCAGA ACTGGGAAAA ATTGATCTTG CCAGCTGTAA
1381 TTCATGGTAG AAAAGAAGTG CTCAGGCTAC TTTTCAACAA AGGAGCAGAT GTAAACTACA
1441 TCTTTGAAAG AAATGGAAAA TCATATACTG TTTTGGAATT GATTAAAGAA AGTTACTCTG
1501 AGACACAAAA GAGGTAGCTG AAGTGGTACT CTCAAAATGC AGAACGATGA CTGCGAAGCA
1561 AGAAGTAGAG AAATAACACT TTATGACTTT CTTAGTTGTA GAAAGATAG AGATATAATG
1621 ATGGTCATAA ATAACTCTGA TATTGCAAGT AAATGCAATA ATAAGTTAGA TTTATTTAAA
1681 AGGATAGTTA AAAATAGAAA AAAAGAGTTA ATTTGTAGGG TTAAAATAAT ACATAAGATC
1741 TTAAAATTTA TAAATACGCA TAATAATAAA AATAGATTAT ACTTATTACC TTCAGAGATA
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Figure 10 (cont'd.)

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1801 AAATTTAAGA TATTTACTTA TTTAACTTAT AAAGATCTAA AATGCATAAT TTCTAAATAA
1861 TGAAAAAAAG TACATCATGA GCAACGCGTT AGTATATTTT ACAATGGAGA TTAACGCTCT
1921 ATACCGTTCT ATGTTTATTG ATTCAGATGA TGTTTTAGAA AAGAAAGTTA TTGAATATGA
1981 AAACTTTAAT GAAGATGAAG ATGACGACGA TGATTATTGT TGTAAATCTG TTTTAGATGA
2041 AGAAGATGAC GCGCTAAAGT ATACTATGGT TACAAAGTAT AAGTCTATAC TACTAATGGC
2101 GACTTGTGCA AGAAGGTATA GTATAGTGAA AATGTTGTTA GATTATGATT ATGAAAAACC
2161 AAATAAATCA GATCCATATC TAAAGGTATC TCCTTTGCAC ATAATTTCAT CTATTCCTAG
2221 TTTAGAATAC TTTTCATTAT ATTTGTTTAC AGCTGAAGAC GAAAAAAATA TATCGATAAT
2281 AGAAGATTAT GTTAACTCTG CTAATAAGAT GAAATTGAAT GAGTCTGTGA TAATAGCTAT
2341 AATCAGAGAA GTTCTAAAAG GAAATAAAAA TCTAACTGAT CAGGATATAA AAACATTGGC
2401 TGATGAAATC AACAAGGAGG AACTGAATAT AGCTAAACTA TTGTTAGATA GAGGGGCCAA
2461 AGTAAATTAC AAGGATGTTT ACGGTTCTTC AGCTCTCCAT AGAGCTGCTA TTGGTAGGAA
2521 ACAGGATATG ATAAAGCTGT TAATCGATCA TGGAGCTGAT GTAAACTCTT TAACTATTGC
2581 TAAAGATAAT CTTATTAAAA AAAAATAATA TCACGTTTAG TAATATTAAA ATATATTAAT
2641 AACTCTATTA CTAATAACTC CAGTGGATAT GAACATAATA CGAAGTTTAT ACATTCTCAT
2701 CAAAATCTTA TTGACATCAA GTTAGATTGT GAAAATGAGA TTATGAAATT AAGGAATACA
2761 AAAATAGGAT GTAAGAACTT ACTAGAATGT TTTATCAATA ATGATATGAA TACAGTATCT
2821 AGGGCTATAA ACAATGAAAC GATTAAAAAT TATAAAAATC ATTTCCCTAT ATATAATACG
2881 CTCATAGAAA AATTCATTTC TGAAAGTATA CTAAGACACG AATTATTGGA TGGAGTTATA
2941 AATTCTTTTC AAGGATTCAA TAATAAATTG CCTTACGAGA TTCAGTACAT TATACTGGAG
3001 AATCTTAATA ACCATGAACT AAAAAAAATT TTAGATAATA TACATTAAAA AGGTAAATAG
3061 ATCATCTGTT ATTATAAGCA AAGATGCTTG TTGCCAATAA TATACAACAG GTATTTGTTT
3121 TTATTTTTAA CTACATATTT GATGTTCATT CTCTTTATAT AGTATACACA GAAAATTCAT
3181 AATCCACTTA GAATTTCTAG TTATCTAG
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Figure 11

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   1 AAGCTTCTAT CAAAAGTCTT AATGAGTTAG GTGTAGATAG TATAGATATT ACTACAAAGG
  61 TATTCATATT TCCTATCAAT CTAAAGTAG ATGATATTAA TAACTCAAAG ATGATGATAG
 121 TAGATAATAG ATACGCTCAT ATAATGACTG CAAATTTGGA CGGTTCACAT TTTAATCATC
 181 ACGCGTTCAT AAGTTTCAAC TGCATAGATC AAAATCTCAC TAAAAAGATA GCCGATGTAT
 241 TTGAGAGAGA TTGGACATCT AACTACGCTA AGAAATTAC AGTTATAAAT AATACATAAT
 301 GGATTTTGTT ATCATCAGTT ATATTAACA TAAGTACAAT AAAAAGTATT AAATAAAAAT
 361 ACTTACTTAC GAAAAAATGT CATTATTACA AAAACTATAT TTTACAGAAC AATCTATAGT
 421 AGAGTCCTTT AAGAGTTATA ATTTAAAAGA TAACCATAAT GTAATATTTA CCACATCAGA
 481 TGTTGATACT GTTGTAGTAA TAAATGAAGA TAATGTACTG TTATCTACAA GATTATTATC
 541 ATTTGATAAA ATTCTGTTTT TTAACTCCTT TAATAACGGT TTATCAAAAT ACGAAACTAT
 601 TAGTGATACA ATATTAGATA TAGATACTCA TAATTATTAT ATACCTAGTT CTTCTTCTTT
 661 GTTAGATATT CTAAAAAAAA GAGCGTGTGA TTTAGAATTA GAAGATCTAA ATTATGCGTT
 721 AATAGGAGAC AATAGTAACT TATATTATAA AGATATGACT TACATGAATA ATTGGTTATT
 781 TACTAAAGGA TTATTAGATT ACAAGTTTGT ATTATTGCGC GATGTAGATA AATGTTACAA
 841 ACAGTATAAT AAAAAGAATA CTATAATAGA TATAATACAT CGCGATAACA GACAGTATAA
 901 CATATGGGTT AAAAATGTTA TAGAATACTG TTCTCCTGGC TATATATTAT GGTTACATGA
 961 TCTAAAAGCC GCTGCTGAAG ATGATTGGTT AAGATACGAT AACCGTATAA ACGAATTATC
1021 TGCGGATAAA TTATACACTT TCGAGTTCAT AGTTATATTA GAAAATAATA TAAAACATTT
1081 ACGAGTAGGT ACAATAATTG TACATCCAAA CAAGATAATA GCTAATGGTA CATCTAATAA
1141 TATACTTACT GATTTTCTAT CTTACGTAGA AGAACTAATA TATCATCATA ATTCATCTAT
1201 AATATTGGCC GGATATTTTT TAGAATTCTT TGAGACCACT ATTTTATCAG AATTTATTTC
1261 TTCATCTTCT GAATGGGTAA TGAATAGTAA CTGTTTAGTA CACCTGAAAA CAGGGTATGA
1321 AGCTATACTC TTTGATGCTA GTTTATTTTT CCAACTCTCT ACTAAAAGCA ATTATGTAAA
1381 ATATTGGACA AAGAAAACTT TGCAGTATAA GAACTTTTTT AAAGACGGTA AACAGTTAGC
1441 AAAATATATA ATTAAGAAAG ATAGTCAGGT GATAGATAGA GTATGTTATT TACACGCAGC
1501 TGTATATAAT CACGTAACTT ACTTAATGGA TACGTTTAAA ATTCCTGGTT TTGATTTTAA
1561 ATTCTCCGGA ATGATAGATA TACTACTGTT TGGAATATTG CATAAGGATA ATGAGAATAT
1621 ATTTTATCCG AAACGTGTTT CTGTAACTAA TATAATATCA GAATCTATCT ATGCAGATTT
1681 TTACTTTATA TCAGATGTTA ATAAATTCAG TAAAAAGATA GAATATAAAA CTATGTTTCC
1741 TATACTCGCA GAAAACTACT ATCCAAAAGG AAGGCCCTAT TTTACACATA CATCTAACGA
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Figure 11 (cont'd.)

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1801 AGATCTTCTG TCTATCTGTT TATGCGAAGT AACAGTTTGT AAAGATATAA AAAATCCATT
1861 ATTATATTCT AAAAAGGATA TATCAGCAAA ACGATTCATA GGTTTATTTA CATCTGTCGA
1921 TATAAATACG GCTGTTGAGT AAGAGGATA TAAAATAAGA GTAATAGGAT GTTTAGAATG
1981 GCCTGAAAAG ATAAAAATAT TTAATTCTAA TCCTACATAC ATTAGATTAT TACTAACAGA
2041 AAGACGTTTA GATATTCTAC ATTCCTATCT GCTTAAATTT AATATAACAG AGGATATAGC
2101 TACCAGAGAT GGAGTCAGAA ATAATTTACC TATAATTTCT TTTATCGTCA GTTATTGTAG
2161 ATCGTATACT TATAAATTAC TAAATTGCCA TATGTACAAT TCGTGTAAGA TAACAAAGTG
2221 TAAATATAAT CAGGTAATAT ATAATCCTAT ATAGGAGTAT ATATAATTGA AAAAGTAAAA
2281 ATAAATCATA TAATAATGAA ACGAAATATC AGTAATAGAC AGGAACTGGC AGATTCTTCT
2341 TCTAATGAAG TAAGTACTGC TAAATCTCCA AAATTAGATA AAAATGATAC AGCAAATACA
2401 GCTTCATTCA ACGAATTACC TTTTAATTTT TTCAGACACA CCTTATTACA AACTAACTAA
2461 GTCAGATGAT GAGAAAGTAA ATATAAATTT AACTTATGGG TATAATATAA TAAAGATTCA
2521 TGATATTAAT AATTTACTTA ACGATGTTAA TAGACTTATT CCATCAACCC CTTCAAACCT
2581 TTCTGGATAT TATAAAATAC CAGTTAATGA TATTAAAATA GATTGTTTAA GAGATGTAAA
2641 TAATTATTTG GAGGTAAAGG ATATAAAATT AGTCTATCTT TCACATGGAA ATGAATTACC
2701 TAATATTAAT AATTATGATA GGAATTTTTT AGGATTTACA GCTGTTATAT GTATCAACAA
2761 TACAGGCAGA TCTATGGTTA TGGTAAAACA CTGTAACGGG AAGCAGCATT CTATGGTAAC
2821 TGGCCTATGT TTAATAGCCA GATCATTTTA CTCTATAAAC ATTTTACCAC AAATAATAGG
2881 ATCCTCTAGA TATTTAATAT TATATCTAAC AACAACAAAA AAATTTAACG ATGTATGGCC
2941 AGAAGTATTT TCTACTAATA AAGATAAAGA TAGTCTATCT TATCTACAAG ATATGAAAGA
3001 AGATAATCAT TTAGTAGTAG CTACTAATAT GGAAAGAAAT GTATACAAAA ACGTGGAAGC
3061 TTTTATATTA AATAGCATAT TACTAGAAGA TTTAAAATCT AGACTTAGTA TAACAAAACA
3121 GTTAAATGCC AATATCGATT CTATATTTCA TCATAACAGT AGTACATTAA TCAGTGATAT
3181 ACTGAAACGA TCTACAGACT CAACTATGCA AGGAATAAGC AATATGCCAA TTATGTCTAA
3241 TATTTTAACT TTAGAACTAA AACGATTCTA CCAATACTAA AAATAGGATA CGTGATAGGC
3301 TGTTAAAAGC TGCAATAAAT AGTAAGGATG TAGAAGAAAT ACTTTGTTCT ATACCTTCGG
3361 AGGAAAGAAC TTTAGAACAA CTTAAGTTTA ATCAAACTTG TATTTATGAA CACTATAAAA
3421 AAATTATGGA AGATACAAGT AAAAGAATGG ATGTTGAATG TCGTAGTTTA GAACATAACT
3481 ATACGGCTAA CTTATATAAA GTGTACGGAC AAAACGAATA TATGATTACT TATATACTAG
3541 CTCTCATAAG TAGGATTAAT AATATTATAG AAACTTTAAA ATATAATCTG GTGGGGCTAG
```

Figure 11 (cont'd.)

3601 ACGAATCTAC AATACGTAAT ATAAATTATA TAATTTCACA AAGAACAAAA AAAAATCAGT

3661 TTCTAATACC TTATAGATAA ACTATATTTT TTACCACTGA CAACAC

| A. | CLASSIFICATION OF SUBJECT MATTER |
|---|---|

IPC(6)   :Please See Extra Sheet.

US CL   :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

| B. | FIELDS SEARCHED |
|---|---|

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   530/333, 388.3, 389.1, 403; 435/69.1, 172.1, 320; 424/184.1, 199.1, 204.1,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN, CABA, MEDLINE, BIOSIS

| C. | DOCUMENTS CONSIDERED TO BE RELEVANT |
|---|---|

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | EP 0 376 744 A1 (CALIFORNIA BIOTECHNOLOGY, INC.) 04 July 1990 (04.07.90), see entire document, especially pages 2-5. | 1, 6-10, 12, 14-17<br>---<br>2-5 |
| X | EP 0 264 979 A1 (DUPHAR INTERNATIONAL RESEARCH B.V) 27 April 1988 (27.04.88), see entire document. | 7-10 |
| Y | VENNEMA et al. Primary Structure of the Membrane and Nucleocapsid Protein Genes of Feline Infectious Peritonitis Virus and Immunogenicity of Recombinant Vaccinia Viruses in Kittens. Virology. 1991, Vol. 181, pages 327-335, see entire document, especially abstract. | 1, 4-6, 11-12, 14-16 |

[X] Further documents are listed in the continuation of Box C.     [ ] See patent family annex.

| * | Special categories of cited documents: | 'T' | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
|---|---|---|---|
| "A" | document defining the general state of the art which is not considered to be of particular relevance | | |
| "E" | earlier document published on or after the international filing date | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | "&" | document member of the same patent family |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 13 MARCH 1997 | 1 4 APR 1997 |

| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231 | Authorized officer   DANNY LEE |
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| Facsimile No.   (703) 305-3230 | Telephone No.   (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)★

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|---|---|---|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| Y | WO 89/03429 A1 (HEALTH RESEARCH INC.) 20 April 1989 (20.04.89), see entire document, especially abstract and page 10. | 1-3 |
| X<br>---<br>Y | TARTAGLIA et al. NYVAC: A Highly attenuated Strain of Vaccinia Virus. Virology. 1992, Vol. 188, pages 217-232, see entire document, especially figure 1, abstract, page 219. | 2-3, 12-13<br>---<br>1-17 |
| Y | TARTAGLIA et al. Protection of Cats against Feline Leukemia Virus by Vaccination with a Canarypox Virus Recombinant, ALVAC-FL. Journal of Virology. April 1993, Vol. 67, No. 4, pages 2370-2375, see entire document. | 1-3, 14-17 |
| A | TARTAGLIA et al. IX Live Vectors as Vaccines: Highly Attenuated Poxvirus Vectors. AIDS Research and Human Retroviruses. 1992, Vol. 8, No. 8, pages 1445-1447, see entire document. | 1-17 |
| A | PICCINI et al. The Use of Vaccinia Virus for the Construction of Recombinant Vaccines. BioEssays. December 1986, Vol. 5, No. 6, pages 248-252, see entire document. | 1-17 |
| A | TAYLOR et al. Fowlpox virus as a vector in non-avian species. Vaccine. December 1988, Vol. 6, pages 466-467, see entire document. | 1-17 |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/19274

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**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**  ☐ The additional search fees were accompanied by the applicant's protest.
                       ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)★

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C12N 15/63, 15/00, 15/09; A61K 39/12, 39/275, 39/285, 39/395, 39/42; C07K 16/08

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/333, 388.3, 389.1, 403; 435/69.1, 172.1, 320; 424/184.1, 199.1, 204.1,

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed
to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1.
The species are as follows:

1. Recombinant poxvirus containing M gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is Vaccinia.
2. Recombinant poxvirus containing M gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is canarypox.
3. Recombinant poxvirus containing the N gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is Vaccinia.
4. Recombinant poxvirus containing S gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is Vaccinia.
5. Recombinant poxvirus containing S1 gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is Vaccinia.
6. Recombinant poxvirus containing S2 gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is Vaccinia.
7. Recombinant poxvirus containing S3 gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is Vaccinia.
8. Recombinant poxvirus containing M+N gene from feline peritonitis virus (FIPV)in a nonessential region wherein
the poxvirus is Vaccinia.
9. Recombinant poxvirus containing S gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is canarypox.
10. Recombinant poxvirus containing S1 gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is canarypox.
12. Recombinant poxvirus containing S2 gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is canarypox.
13. Recombinant poxvirus containing S3 gene from feline peritonitis virus (FIPV)in a nonessential region wherein the
poxvirus is canarypox.
14. Recombinant poxvirus containing M+N gene from feline peritonitis virus (FIPV)in a nonessential region wherein
the poxvirus is canarypox.

The claims are deemed to correspond to the species listed above in the following manner:

1. claims 1, 4-5, 12, 14-17.
2. claims 1, 2-5, 13-17.
3. claims 1, 4-5, 12, 14-17 and 6.
4. claims 1, 4-5, 12, 14-17 and 7.
5. claims 1, 4-5, 12, 14-17 and 8.
6. claims 1, 4-5, 12, 14-17 and 9.
7. claims 1, 4-5, 12, 14-17 and 10.
8. claims 1, 4-5, 12, 14-17 and 11.
9. claims 1, 2-5, 13-17 and 6.
10. claims 1, 2-5, 13-17 and 7.
11. claims 1, 2-5, 13-17 and 8.
12. claims 1, 2-5, 13-17 and 9.
13. claims 1, 2-5, 13-17 and 10.
14. claims 1, 2-5, 13-17 and 11.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The special technical feature of each species is lacking because of the location in the genome, immunological response, primary, secondary and tertiary structures, host response and effector cells.

California Biotechnology Inc. (EP 0376744) teaches the construction of a plasmid insertion vector containing heterologous FIPV gene downstream from vaccinia viral promoter all of which is inserted into the vaccinia thymidine kinase (tk) gene (page 4, lines 29-31), the expression of FIPV proteins, production of FIPV antibodies, production of FIPV proteins in tissue culture and use a living virus immunogen in cats (page 4, lines 41-44).